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EDITED BY

W. B. BRIERLEY

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D. WARD CUTLER

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EDITED FOR THE ASSOCIATION OF ECONOMIC BIOLOGISTS

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FIELD EXPERIMENTS ON THE DETERIORATION OF SCOTCH POTATO SEED IN ENGLAND

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(With 3 Text-figures.)

INTRODUCTION.

THE modern view of the deterioration, *i.e.* loss of yielding power, of potato stocks is that it is the result of infection with various types of virus disease (mosaic, leaf-roll, crinkle, streak, etc.). Numerous workers have brought forward evidence in support of this view, but particular mention should be made of the work of Schultz and Folsom in America, of Quanjer and Botjes in Holland, and of Murphy in Ireland. This theory of potato deterioration—both as regards stocks in a particular locality and as regards the total stock of a particular variety—stands in opposition to the older theory in which deterioration is ascribed to purely physiological causes. This physiological deterioration has been supposed to be brought about in various ways. It is suggested that the potato, being essentially a plant of cold climates, exhibits a disordered metabolism when grown under warm conditions, with the result that deterioration ensues. Hence it is necessary to import fresh, more vigorous stock from colder latitudes. A refinement of this theory is that deterioration is due to the over-ripeness of the "seed" of plants grown under warm conditions, with the corollary that it is possible to check the onset of deterioration by the use of early-lifted, *i.e.* immature seed. It is well known in fact that under English conditions, early-lifted seed in many cases gives a heavier yield than does late-lifted seed from the same stock. It should be noted, however, that the greater potency of early-lifted seed is easily explicable on the disease theory of deterioration inasmuch as the early-lifted seed escapes the infection which takes place during the later part of the growing season, and therefore gives a progeny freer from disease than does the late-lifted seed.

The aim of the work to be described in this paper was to carry out a series of carefully controlled weight tests over a period of years, with a

view to furnishing evidence bearing on the nature of potato deterioration. More particularly, it was proposed to examine what effect immaturity of seed (as obtained by early lifting) had on cropping power. It is clear that the ideal arrangement in such a programme would be to grow potatoes free from virus disease—and to keep them free—over a number of years in England under a variety of physiological conditions, and to use material so derived for inter-comparison and for comparison with healthy material newly imported from the North. Such a scheme is, however, very difficult of realisation. Even the best of seed material commercially available is not completely free from virus disease of one kind or another. Nor was it always possible under the conditions of this work to ensure adequate segregation of the experimental plots from highly infected material in the neighbourhood. It is not surprising that virus disease made its appearance, in some cases more prominently than in others. It was impossible, therefore, to study the problem in the clear-cut manner indicated above; nevertheless, even with virus disease present, certain broad conclusions could be drawn, as is shown below.

General plan of experiment.

Seed material.

Seed of high quality was obtained from the north of Scotland (Nairn) and planted in three localities in England, viz. at the Rothamsted Experimental Station, Harpenden, Herts, at the South-eastern Agricultural College, Wye, Kent, and at the Harper-Adams Agricultural College, Newport, Shropshire. Three varieties were grown at each place—Great Scot, Kerr's Pink and King Edward.

The experiment was begun in the spring of 1922 and was planned in the first instance to run for a period of three years. The three-year period was chosen as it is a common practice in English potato-growing districts to use home-grown seed which is at most one year removed from Scotland. General experience is, therefore, that deterioration sets in during the course of the third season's growth.

The procedure was the same for all three varieties and was substantially the same at all three places. It will be sufficient to describe the procedure at one place, *e.g.* that at the Wye College.

A compact plot (about $\frac{1}{3}$ acre) was planted with freshly imported Scotch seed of the three varieties in the spring of 1922. From this plot seed was lifted at three stages of maturity: (1) very early, (2) medium early, and (3) at the normal time. The first lifting was made at about the

time when tubers of normal seed-size (seven to the pound) could be obtained from the young plants. Generally speaking it was not practicable to ensure that all of the very early-lifted seed was of normal seed size, so that the average seed of this type was distinctly small. The medium early seed was lifted about a month later than the preceding, and about two months in advance of the normal lifting. The actual dates for the plots at Wye were:

Date of planting	May 1st
Very early lifting	July 17th
Medium early lifting	August 16th
Normal lifting	October 31st

An additional experiment was designed with a view to testing the effect of reduced insolation on the growing plants. For this purpose a limited number of plants (*circa* 30) of each variety were grown under a screen. The screening material, which was a light white canvas, was supported on the top of a wooden framework, and it was also carried down 9 inches on all sides. Apart from the uppermost 9 inches, the sides and ends of the framework were open. The height of the framework when in position was about 4 feet, so that the growth of the plants was not restricted. The position of the screen was such that the plants beneath it were protected from direct sunlight during the hotter hours of the day. The shaded plants thus grew under conditions of reduced sunlight and with the soil at a lower temperature than usual, *i.e.* conditions which tended to approximate to those obtaining in Scotland. These plants, as might be expected, showed a somewhat drawn appearance and were definitely later in ripening. It is of interest also to record that they were less attacked by blight than were plants of the same variety growing in the open close by. This effect may have been due to the later ripening of the screened plants, or to the latter being protected from dews and light rains. It may also be that the screens to some extent prevented the deposition of spores of the blight fungus. Seed from the screened plants was lifted in all cases at the time of normal lifting.

Thus at the end of the 1922 season the types of seed saved were as follows:

Very early lifted	$= VE_1$
Medium early lifted	$= ME_1$
Lifted at normal time	$= N_1$
Screened (and lifted at normal time)	$= Sr_1$

The suffix indicates that each type had been grown one year in England.

In 1923 a fresh importation of Scotch seed, from the same district in Northern Scotland, was made and planted out alongside the seed types listed above. A certain number of yield tests were made (see later), but the greater part of the material was reserved for seed for the following year. The VE_1 material was again lifted very early, the ME_1 was lifted medium early, and the Sr_1 type was again grown under a screen and lifted at the normal time. The types of seed available at the end of 1923 were therefore as follows: VE_2, ME_2, Sr_2, N_2 and N_1, the last being the produce of the fresh Scotch seed which was imported in the spring of 1923. The suffixes, as before, indicate the number of years that the particular seed had been grown in England.

In 1924 an elaborate weight test was carried out. A further importation of Scotch seed was made as before, so that the full list of types tested was as follows:

S, fresh Scotch seed;

N_1, one year grown in England and lifted at normal time;

N_2, Sr_2, VE_2, ME_2, all grown two years in England and respectively lifted at normal time throughout, screened and lifted at normal time throughout, lifted very early throughout, and lifted medium early throughout.

At Rothamsted and Harper-Adams College, the same plan was adopted with the simplification that at the former place only one early lifting was made, and at the latter the screening experiment was omitted.

Lay-out of field tests.

For weighing purposes, the standard "plot" consisted of twenty plants spaced evenly along 25 feet of a furrow. The plan of the lay-out will be more clearly understood by reference to Fig. 1 which represents a small central portion of the experimental field at Rothamsted in 1924. In this case fourteen furrows were drawn across the field, and along these the various seed types were planted as indicated in the figure. The number of replications is shown below (*v.* Table II). Rows 1 and 14 were planted with Scotch seed and were used simply as guard rows, *i.e.* the produce of these was not weighed. Gaps of about 1 yard were left between each series of plots, as shown by dotted lines in the figure. The first few yards at the ends of the furrows, where greater mechanical injury was likely to take place in the course of cultivation, were planted up with Scotch seed only and were not included in the test.

The various types of seed followed in succession, as shown in the figure. Some of the types, *e.g.* the screened, and to a less extent the

early-lifted, were available only in limited quantity. When these were exhausted, the replication was continued with the remainder. The comparisons are thus more complete for certain types of seed than for others. The number of such plots in 1924 at Wye when the main test was made was 298. The number at Rothamsted and at Harper-Adams College in the same year was of the same order but somewhat less.

Each plot of twenty plants was lifted, cleaned and weighed separately. In this main test, all the plots were lifted at the normal time.

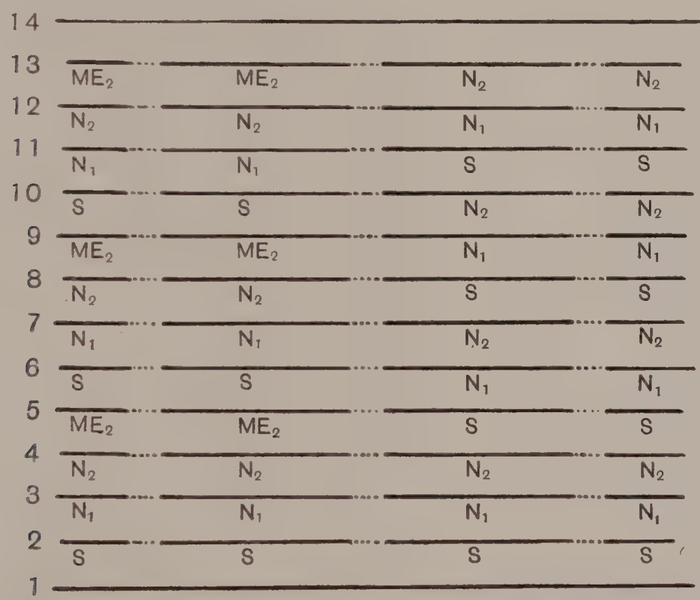

Fig. 1. Figure showing layout of experiment at Rothamsted Experimental Station in 1924; fourteen furrows are shown. S, fresh Scotch seed; N_1, seed grown one year in England and lifted at normal time; N_2, as N_1 but grown two years in England; ME_2, grown two years in England and lifted at a medium early date.

Rogueing. The types of seed which have been described above represent all those which were carried through to the finish of the experiment. To begin with, however, a somewhat wider scope was envisaged, and a test was to be carried out of the effectiveness of a scheme of rogueing in checking the spread of virus diseases over a plot which was liable to infection from diseased material growing in the neighbourhood. For this reason the original mass plots, as planted in 1922, were placed in the neighbourhood of plots containing a high percentage of diseased plants. For example, the plot at Wye was surrounded on three sides by such

highly diseased material. It was proposed therefore to have, in addition to the seed types already mentioned, such further types as (1) seed from the neighbourhood of diseased material, and which had not been rogued, (2) similar seed which had been rogued. It soon became apparent that with the experimental area available, the multiplication of seed types, some of which would be expected to become highly diseased, would defeat the object of the experiment. Accordingly the scheme was soon simplified, but not before a certain amount of otherwise avoidable infection had taken place. The seed that was actually raised in season 1922 was taken from a part as far removed as possible from the surrounding diseased material. The maximum distance was about 20 yards, and in the case of one variety was only 10 yards. It is very doubtful if this degree of isolation can be described as safe. At any rate it is clear that unnecessary risks of deterioration from virus disease infection were run in the first year. In subsequent years contamination from inferior material was avoided as far as circumstances would admit.

In order to check the spread of virus disease among the seed stocks a definite scheme of inspection and rogueing was carried out. The plots were given three rogueings during each growing season, the first when the plants were about 6 inches high, the second about a month later, and the third a month later still. It is obvious that the above cannot be considered a fair test of the effectiveness of rogueing as a means of controlling virus disease. More numerous inspections and rogueings, with a view to eliminating diseased plants as soon as they were apparent, would clearly be more effective.

During the early course of this work, viz. from 1922 to 1924, the aim was to test the effect of various treatments (early lifting, shading) on the yielding capacity of seed. From 1925 to the present date the experiment was continued with a somewhat changed objective, viz. to see how far the yielding power of seed grown in England could be maintained at the standard of Scotch seed by annual rogueing. For the sake of clearness the results obtained in these two tests will be described separately.

Effect of early lifting and shading on the yielding capacity of potato seed.

1. *Rothamsted experiment.*

1922. The plots at this station were planted in the form of a long strip with potatoes along one side and turnips along the other. The seed for the following year was taken from the side of the strip next the turnips.

It is perhaps on account of this original lay-out that virus diseases were more effectively held in check here than at the other two stations.

1923. The seed saved from 1922, viz. N_1, ME_1 and Sr_1, together with fresh S seed, was planted out in the midst of a field of Kerr's Pink potatoes (Scotch seed). The amount of disease shown in the various plots

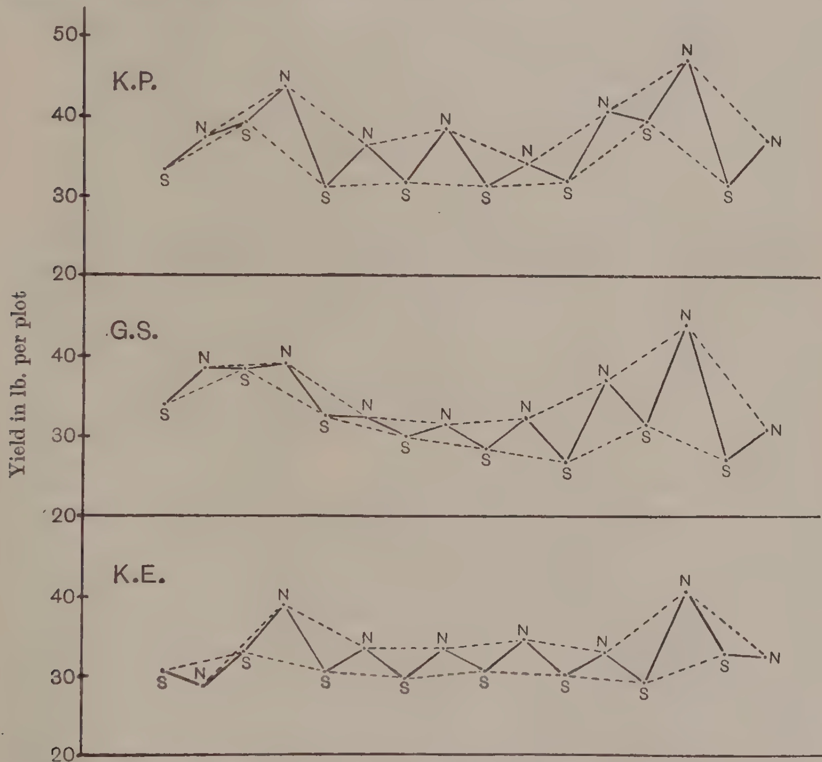

Fig. 2. Three graphs showing the yield (lb. per plot of twenty plants) of Kerr's Pink (K.P.), Great Scot (G.S.) and King Edward (K.E.) at Rothamsted in 1923. For each variety, the yields of eight plots of fresh Scotch seed (S) and of seed grown one year in England and lifted at the normal time (N) are shown; the N and S plots were arranged alternately across the field.

was negligible—viz. out of 2400 plants, three plants showing definite symptoms of leaf-roll were rogued out. These were all present in seed of the N_1 type.

A comparison between S and N_1 seed of the three varieties gave an interesting result. It is commonly stated by potato growers that one-year grown (N_1) seed generally yields a heavier crop than does fresh Scotch seed. Confirmation of this belief is seen in Fig. 2, which represents

the yields (in lb. per plot of twenty plants) of alternating plots of S and N_1 seed of the three varieties. It will be seen that the N_1 plots in nearly every case outweigh the S plots. The result is perhaps most clearly shown by joining up the consecutive S and N_1 points separately, as is done in the figure by the dotted lines. The superiority of the N_1 seed in yielding power over all the S seed is seen inasmuch as the curve joining up the N_1 points lies everywhere above the S curve, with a single exception at the left-hand extremity of the King Edward series.

The statistical data for this comparison are given in Table I below.

Table I.

A comparison of the yielding powers of fresh Scotch (S) and one-year grown (N_1) seed. The mean weight per plot with the probable error is given.

Variety	Type of seed	No. of plots	No. of plants	Av. wt. per plot (lb.)
Great Scot	S	8	157	$31{\cdot}31 \pm 0{\cdot}94$
,,	N_1	8	156	$35{\cdot}88 \pm 1{\cdot}12$
Kerr's Pink	S	8	160	$33{\cdot}75 \pm 0{\cdot}85$
,,	N_1	8	158	$39{\cdot}44 \pm 1{\cdot}02$
King Edward	S	8	157	$30{\cdot}81 \pm 0{\cdot}33$
,,	N_1	8	159	$34{\cdot}44 \pm 0{\cdot}88$

The difference in favour of the one-year grown (N_1) seed is statistically significant for all three varieties.

It should be stated that the N_1 seed had been kept in trays over winter, so that good sprouts were present at the time of planting. The S seed, on the other hand, was only brought south in the spring and planted straight from the bags, so that any sprouts which were present were liable to be damaged or broken off. It is probable that a great deal if not all of the difference observed could be explained on the basis of these different modes of treatment.

The average yield of the forty-eight plots is approximately 34·5 lb. With the spacing adopted this corresponds to a yield of 9·5 tons to the acre, *i.e.* to a good average crop. It may be added that unless in these experiments a good average crop was realised from seed of good quality, no significance was attached to any comparative results that might be obtained.

1924. The types of seed planted were S, N_1, N_2, ME_2 and Sr_2 for each of the three varieties. The fresh Scotch seed (S) was brought down early in the year and sprouted in trays like the remaining types. All the seed was thus well sprouted at the time of planting. The number of plots of each kind is shown in Table II.

Table II.

Showing number of plots of each type of seed (*Rothamsted*, 1924).

Variety	Types of seed: S	N_1	N_2	ME_2	Sr_2
Great Scot	24	24	24	8	2
Kerr's Pink	24	24	24	8	2
King Edward	24	18	24	2	2

Neither the ME_2 nor Sr_2 types of seed could be tested as thoroughly as the others on account of lack of sufficient seed.

The amount of virus disease detected in the plots was comparatively small, and especially so in the Great Scot variety, though it was greater than was anticipated from the almost complete freedom from disease of the plants of the previous year. All the available seed was used up in the elaborate weight test series described above, so that when it was decided during the course of the season to carry on the work for some years longer, a certain amount of rogueing was deemed advisable. This was done at the first inspection (June 25th). At the subsequent inspections further infected plants were noted but not removed. The actual number of plants removed is indicated in the various tables below. The correct procedure, of course, would be to rogue the plots from which the following year's seed was to be obtained and to leave unrogued the plots for the current year's weight test. The plan adopted was in the nature of a compromise, and though it was not ideal it will appear that the conclusions to be drawn from the data were not seriously affected.

For convenience of presentation, only two types of virus disease will be noted, viz. leaf-roll (L.R.) and mosaic type (M.T.). The latter term is to be taken as including mosaic, crinkle, streak—in fact all forms of virus disease other than leaf-roll. This grouping is practically necessary as the synonymy of the virus diseases of potato other than leaf-roll is far from settled. A further difficulty in determining the amount of infestation with virus disease is that the symptoms are often so slight or indefinite that it is difficult to say off-hand whether disease is present or not. Cases of this kind are indicated below by a question mark. It is obvious also that many plants showing slight symptoms of disease would be passed as healthy, especially under the conditions of fairly rapid inspection which was all that was practicable in carrying out the work. Accordingly, the figures given in the tables undoubtedly understate the amount of virus disease actually present. On the other hand, they represent fairly the condition of the plots as regards virus disease in its more definite

forms, and it is probably only in such forms that virus disease seriously affects the yielding capacity of the crop.

The relative infestation with virus disease and the yielding capacity of the various types of seed will now be given for each of the three varieties (Tables III to IX).

Great Scot.

The amount and kind of virus disease present in the various plots are given in Table III. The figures in the last column represent roughly the percentages of *definitely* infected plants.

Table III.

Amount and kind of virus disease present in plots of Great Scot.

Type of seed	No. of plants (approx.)	Disease present	% diseased
S	480	0	0
N_1	480	3 L.R. + 1 ? L.R. + 3 ? M.T.	<1
N_2	480	2 L.R. + 1 ? L.R. + 2 M.T.	<1
ME_2	160	1 L.R. + 2 M.T. + 7 ? M.T.	2
Sr_2	40	0	0

The corresponding yields per plot (except for the screened seed) are given in Table IV.

Table IV.

Yields (with probable errors) of four types of Great Scot seed.

Type of seed	No. of plots	No. of plants	Av. wt. per plot (lb.)
S	24	466 (466)	$31{\cdot}15 \pm 0{\cdot}53$
N_1	24	464 (469)	$32{\cdot}70 \pm 0{\cdot}73$
N_2	24	456 (460)	$30{\cdot}85 \pm 0{\cdot}67$
ME_2	8	155 (155)	$33{\cdot}70 \pm 0{\cdot}92$

The number of plants planted was 480 for the first three types and 160 for the last. The figures in the third column of the above table give the number of plants actually present at lifting time; the figures in brackets give the numbers which would have been present if a certain amount of rogueing had not been carried out. None of the average weights as given in the fourth column differs from any other by an amount which is statistically significant. If a correction is made for the plants rogued out, either by allowing for them the average weight per plant, or what would perhaps be fairer, by allowing half the average weight, the conclusion is unaltered, viz. that there is no significant difference in the average yields of the four types of seed. Furthermore, there is no sug-

gestion in the figures that any of the three types of English-grown seed is yielding less than the Scotch seed.

The plots of screened (Sr_2) seed[1] were too few to allow of statistical treatment of the results, but the following figures show that the screened seed gave a yield practically the same as the N_2 seed growing alongside.

Sr_2 (2 plots). Av. wt. = 29·3 lb.
N_2 (2 plots). ,, = 28·6 ,,

Kerr's Pink.

The prevalence of virus disease was distinctly greater than was the case with the preceding variety. Table V gives a record of the amount and type of disease present.

Table V.

Amount and kind of virus disease present in plots of Kerr's Pink.

Type of seed	No. of plants (approx.)	Diseased plants	% diseased plants
S	480	1 M.T. + 1 ? M.T.	0–1
N_1	480	1 L.R. + 3 ? L.R. + 22 M.T. + 2 ? M.T.	5
N_2	480	6 L.R. + 16 M.T. + 2 ? M.T.	5
ME_2	160	8 M.T.	5
Sr_2	40	1 M.T.	2–3

The corresponding yields per plot (except for the screened seed) are given in Table VI.

Table VI.

Yields (with probable errors) of four types of Kerr's Pink seed.

Type of seed	No. of plots	No. of plants	Av. wt. per plot (lb.)
S	24	469	37·23 ± 0·64
N_1	24	452	32·82 ± 0·55
N_2	24	441	34·54 ± 0·59
ME_2	8	158	37·90 ± 1·30

In this case both the N_1 and N_2 types show a significant falling off in yield compared with the S type. An appreciable number of plants were, however, rogued out in this series, and some allowance should be made for them. On the basis of an allowance of half the average weight per plant for those which were rogued out, the figures are as in Table VII.

As now calculated, the yield of the N_1 type of seed is significantly less than that of the Scotch seed, whereas that of the N_2 is no longer so. Even when full average weight is allowed for the plants rogued out, the difference between the yields of S and N_1 seed is significant.

[1] It is to be noted that this stock was grown under the screen in seasons 1922 and 1923, but that it was planted in the open, side by side with the other types of seed, in 1924.

Table VII.

Corrected yields for four types of Kerr's Pink seed.

Type of seed	No. of plots	No. of plants (corrected)	Av. wt. per plot (lb.)
S	24	471	37·30 ± 0·62
N_1	24	467	33·36 ± 0·54
N_2	24	459	35·27 ± 0·60
ME_2	8	158	37·90 ± 1·30

The figures given in Tables VI and VII indicate a falling off of the N_1 and N_2 types as compared with fresh Scotch seed, though on the data available this deterioration is only statistically significant for the N_1 type of seed. That the N_1 type should apparently deteriorate more than the N_2 type is remarkable, but it is not clear what meaning is to be attached to the apparent difference. It is noteworthy also that the ME_2 type of seed shows no evidence of deterioration even though it contains more or less the same percentage of diseased plants as the N_1 and N_2 types.

The comparison of the screened (Sr_2) seed with the corresponding normal (N_2) seed growing alongside gave the following figures:

Sr_2 (2 plots). Av. wt. = 28·0 lb.
N_2 (2 plots). ,, = 33·3 lb.

The number of plots is too small to allow of the significance of these figures being estimated, but at any rate there is no indication that the protection from high temperatures afforded to the plants in the preceding years has in any way increased their yielding power.

King Edward.

The percentage of plants showing virus disease was fairly similar to that shown by the Kerr's Pink plots. The record of diseased plants is given in Table VIII.

Table VIII.

Amount and kind of virus disease present in plots of King Edward.

Type of seed	No. of plants (approx.)	Diseased plants	% diseased plants
S	480	1 ? M.T.	0
N_1	360	1 L.R. + 16 M.T. + 2 ? M.T.	5
N_2	480	5 L.R. + 23 M.T. + 13 ? M.T.	6
ME_2	40	1 M.T.	2–3
Sr_2	40	2 M.T.	5

The yields of the three types of seed, S, N_1 and N_2, were determined over a series of eighteen plot replications. The results are given in Table IX.

Table IX.

Yields (with probable errors) of three types of King Edward seed.

Type of seed	No. of plots	No. of plants	Av. wt. per plot (lb.)
S	18	349	$29{\cdot}22 \pm 0{\cdot}42$
N_1	18	351	$27{\cdot}83 \pm 0{\cdot}48$
N_2	18	323	$27{\cdot}53 \pm 0{\cdot}72$

Both the English-grown types of seed show a falling off in yield as compared with the Scotch, but in neither case is the drop statistically significant. The yield of the N_2 type is unduly low on account of a somewhat poor stand of plants (from causes unknown) and it is not clear that there is in this case any falling off in yield significant or otherwise. In spite, therefore, of a certain percentage (*circa* 5 per cent.) of virus-infected plants among the English-grown King Edward plants, there is no clear evidence of any deterioration in yielding power.

Only two plots each of the early-lifted (ME_2) and screened (Sr_2) types of seed were available for comparison. The results for these and for the two rows of N_2 seed growing alongside were as follows:

N_2 (2 plots). Av. wt. = 28·0 lb.
ME_2 (2 plots). ,, = 30·8 lb.
Sr_2 (2 plots). ,, = 24·5 lb.

The duplicate plots were not in good agreement so that the significance of these figures is doubtful. As regards the screened seed, however, the figures do not indicate any difference from the behaviour of the other two varieties.

2. *Wye experiment.*

1922. That the plots at Wye in 1922 were exposed to considerable risk of infection has already been stated (p. 6); this is in all probability one of the reasons why the plots at this centre have shown in subsequent years a greater degree of infection with virus disease than the corresponding plots at Rothamsted. The types of seed lifted were the same as at the latter centre, with the addition of a very early type (VE).

1923. The proportion of leaf-rolled plants present in the plots, especially in those of King Edward, was in marked contrast with that shown at Rothamsted. Table X shows the data for the plots of Great Scot and King Edward.

The presence of 3 per cent. of leaf-rolled plants in the plot of Scotch King Edward was very exceptional, the general experience in this work being that the seed from Banffshire was of very high quality.

Table X.

Percentage of leaf-rolled plants in plots of Great Scot and King Edward.

Type of seed	Great Scot	King Edward
S	0·3	3
N_1	2	7
VE_1	2	2
ME_1	1·5	0·6
Sr_1	1	3

The plots of Kerr's Pink were practically free of leaf-roll, there being only two diseased plants in the whole series of plots of this variety.

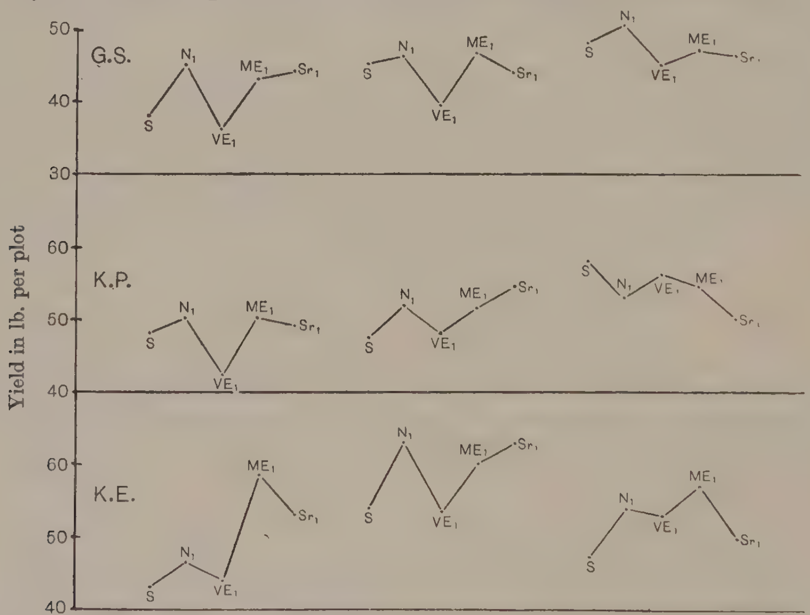

Fig. 3. Graphs showing yields of Great Scot, Kerr's Pink, and King Edward potatoes in 1923 at Wye. S and N, as in Fig. 2; VE_1, seed lifted very early and grown one year in England; ME_1, seed lifted medium early and grown one year in England; Sr_1, seed from screened plants grown one year in England.

An unfortunate circumstance in the history of these plots was that there was considerable intermixture with self-sown rogues over a certain area of the plots. Care was always taken in this work that the potatoes did not succeed a potato crop of the preceding year, but in this instance the presence of rogues was found to be due to potato fragments among the farmyard manure used. Practically all these rogue plants (fifty-two altogether) showed some form or other of virus disease. They were

removed at the first inspection (June 22nd). At this date only few aphides were observed, but nevertheless it is not improbable that some infection had already taken place.

As the majority of the material was reserved for seed for the following year only a limited number of weight tests was carried out. The data are too few to warrant statistical treatment, but certain conclusions are indicated, as may be seen from Fig. 3. If we consider the three varieties together, it is seen that out of nine comparisons between S and N_1 plots, the yield of the latter exceeds that of the former in eight cases. This is a further indication of the rule found to apply at Rothamsted, that the one-year grown seed yields somewhat more heavily than fresh Scotch seed. A second feature, also shown in eight cases out of nine, is the dip in the curves at the point corresponding to the very early lifted (VE_1) type of seed. Though this is the only occasion on which this effect has been observed, the agreement among the curves indicates that very early lifted seed may be inferior to late-lifted seed, even though it may contain a smaller percentage of diseased plants (see figures for King Edward variety in Table X). The very early lifted seed in this case was lifted on July 17th and was thus very immature. The result of this test so far as it goes thus supports the view of Murphy and McKay[1], which is that very immature seed, in the absence of virus disease, is intrinsically inferior to seed lifted at the normal time.

1924. On account of the considerable infestation with virus disease which became obvious during the growing season, the weight test plots were not rogued, but a number of plots, which had originally been laid out for weighing, were severely rogued and used solely for the supply of seed for the following year.

In the Great Scot variety, about 5 per cent. of leaf-rolled plants were present in the plots of one- and two-year English-grown material. The other two varieties showed somewhat more, up to 10 per cent. in some plots. The commonest type of virus disease was, however, of the mosaic type, viz. a kind of streak[2]. This disease was undoubtedly present in a mild form in the preceding year, but had now become much more widely spread and produced more distinct symptoms on the attacked plants.

[1] Murphy, P. A. and McKay, R., "Investigations on the Leaf Roll and Mosaic Diseases of the Potato." *Journ. of Dept. of Lands and Agric., Irish Free State,* xxv, No. 2, 18.

[2] The type of streak is that known as "leaf-drop" streak. It is not the "stipple streak" of Atanosoff (*Meded. Landbouwhoogeschool, Wageningen,* xxiv, No. 5, p. 32, 1922). The affected plants do not succumb to the disease in the manner characteristic of "stipple streak." "Leaf-drop" streak and its relation to other virus diseases, such as mosaic and crinkle, are far from clear. Compare footnote on p. 20.

The general effect of this disease being to cause the premature death of the haulms, a rough idea of its prevalence was obtainable by counting towards the end of the season the number of prematurely ripened plants. A sample set of figures, obtained in this way, is shown in Table XI.

Table XI.

Percentage of fully ripened plants on September 9th.

	% of ripened plants	
Seed type	Great Scot	Kerr's Pink
S	1	2
N_1	40	54
N_2	32	44
VE_2	11	47
ME_2	25	68
Sr_2	30	55

It is not certain that all the faded plants were infected with virus disease, though undoubtedly most of them were so. Table XI clearly shows how all the English types of seed showed up badly in this respect when compared with the fresh Scotch seed. A general review of all the plots of all the varieties showed that the VE_2 type of seed was certainly less infected than any of the other English-grown types, but the difference was not always clearly marked.

The results of the weight tests are given in Tables XII, XIV and XV below.

Table XII.

Yields (with probable errors) of six types of Great Scot seed.

Seed type	No. of plots	No. of plants	Av. wt. per plot (lb.)
S	11	216	$41{\cdot}8 \pm 1{\cdot}40$
N_1	11	205	$31{\cdot}5 \pm 1{\cdot}10$
N_2	11	201	$29{\cdot}0 \pm 0{\cdot}97$
VE_2	10	199	$38{\cdot}8 \pm 1{\cdot}59$
ME_2	10	197	$32{\cdot}7 \pm 1{\cdot}38$
Sr_2	6	113	$31{\cdot}7 \pm 1{\cdot}18$

Calculation from the data of Table XII shows that definite deterioration has taken place in the N_1, N_2, ME_2 and Sr_2 types of seed as compared with that fresh from Scotland. The yield of the VE_2 type of seed is also less than that of the Scotch, but the difference is not significant. On the other hand, the yield of the VE_2 seed is significantly greater than that of the N_2 type, thus proving in this case the advantage derived from early lifting.

An examination of the figures in the third column of Table XII and of Tables XIV and XV below shows that the number of plants present at harvesting time relative to the number planted is less for the seed types N_1, N_2 and Sr_2 than for the other three types[1]. The data are brought together in Table XIII.

Table XIII.

Percentage "germination" of different seed types.

Seed type	Great Scot	Kerr's Pink	King Edward
S	98·2	98·0	100
N_1	93·2	90·9	91·8
N_2	91·4	90·0	88·6
VE_2	99·5	98·3	99·5
ME_2	98·5	98·9	97·3
Sr_2	94·1	88·8	80·0

The cause of the higher percentage of "misses" among the late-lifted English types was probably the presence of the potato blight fungus on the sets. When a correction is made for this irregularity of sprouting by giving full value to the missing plants, the conclusions derived from Table XII above and Tables XIV and XV below are unaffected.

Kerr's Pink.

Table XIV.

Yields (with probable errors) of six types of Kerr's Pink seed.

Seed type	No. of plots	No. of plants	Av. wt. per plot (lb.)
S	12	235	$41·2 \pm 1·22$
N_1	11	200	$30·0 \pm 0·99$
N_2	11	197	$29·7 \pm 1·16$
VE_2	9	177	$34·1 \pm 1·38$
ME_2	9	178	$30·8 \pm 1·54$
Sr_2	8	142	$29·0 \pm 1·78$

Compared with the fresh Scotch seed, all the types of English-grown seed have definitely deteriorated. The VE_2 type, though better than the corresponding late-lifted (N_2) type, is not significantly so. The types N_1, N_2, ME_2 and Sr_2 show negligible differences. The general result is thus similar to that shown by the Great Scot series, except that deterioration is somewhat more pronounced. Thus, in contrast with the results for the first

[1] The number of plants planted is obtained by multiplying the number of plots as given in column 2 by 20.

variety, the VE_2 type of seed shows definite deterioration as compared with Scotch seed, and is not significantly different from late-lifted seed (N_2) which has been grown for the same period of time in England.

King Edward.

Table XV.

Yields (with probable errors) of six types of King Edward seed.

Seed type	No. of plots	No. of plants	Av. wt. per plot (lb.)
S	12	240	$32{\cdot}7 \pm 1{\cdot}34$
N_1	11	202	$24{\cdot}4 \pm 1{\cdot}11$
N_2	11	195	$19{\cdot}0 \pm 1{\cdot}15$
VE_2	11	219	$28{\cdot}5 \pm 1{\cdot}78$
ME_2	11	214	$24{\cdot}3 \pm 1{\cdot}24$
Sr_2	4	64	$24{\cdot}0 \pm 0{\cdot}42$

Compared with Scotch seed, all the English types except VE_2 show significant deterioration. The VE_2 type is not significantly different from the S type, but is significantly greater than the N_2 type. The results are thus similar to those obtained for the variety Great Scot.

In carrying out the above weight tests at Wye in the season 1924, certain observations were made which in themselves indicate whether virus disease or the direct influence of climate is responsible for loss of cropping power. If deterioration were due to climatic influence *per se* one would expect to find evidence of some general physiological change more or less uniformly marked in all the plants which had been under the same conditions. Thus the deteriorated N_2 plots would be expected to show some fairly uniform reduction in size of the haulms. This general reduction in haulm would then be associated with a general tendency to poor yield in each plant. This, however, is not the case. The plots which gave poor yields did so, not because each plant was slightly deteriorated, but because the plots were composed of a mixture of normal plants giving normal yields and highly deteriorated plants giving very small yields. The discontinuous nature of the degeneration effect obviously does not fit in with the physiological theory of deterioration, while it is just what would be expected on the disease theory.

The feature just referred to is well brought out in Table XVI, which is based on separate weighings of the healthy and diseased plants in a number of deteriorated plots of the Kerr's Pink variety. The plants which showed no symptoms of virus disease in their haulms and those which did were marked, and at lifting time the two lots were weighed separately.

Table XVI.

Average yield per root of healthy and diseased plants of Kerr's Pink.

Type of seed	Plants in plot	Healthy	Av. wt. (lb.)	Diseased	Av. wt. (lb.)
N_1	19	14	1·96	5	1·05
N_1	19	14	2·32	5	1·05
N_1	15	8	2·25	7	0·90
N_2	16	11	2·50	5	1·20
N_2	20	13	2·54	7	0·36
ME_2	20	11	2·00	9	0·72
ME_2	20	11	2·32	9	1·10

The average weight per root for the diseased plants is 0·91 lb. as against 2·27 lb. for the healthy. The latter figure is fully equal to that of the Scotch plots of this variety.

Harper-Adams experiment.

The experiment at Harper-Adams College for the period 1922–4 ran a course fairly similar to that described for the experiment at Wye. A good deal of disease appeared, partly on account of risks which were run in the first year, and partly because the programme of rogueing could not be adhered to satisfactorily. The records of the various plots in season 1923 are very similar to those already given for the Wye experiment, and as the main weight test laid out in 1924 proved to be an utter failure, it is unnecessary to reproduce the details. In season 1924 the experimental field suffered from a heavy flooding in middle summer and the whole crop was ruined. Weighings were carried out in the autumn as usual, but so poor was the crop that even the plots from fresh Scotch seed only yielded at the rate of 1 to 2 tons per acre. The weighings were therefore quite valueless.

Continuation of the experiment.

From 1925 onward the work was continued in a simplified form and, as has already been mentioned, with a somewhat changed objective. By the end of 1924 all the stocks had in greater or less degree become infected with virus disease. The experiment was therefore continued with the object of seeing how far rogueing was effective in eliminating disease, or failing elimination, in preventing further deterioration. Only the late-lifted seed (excluding screened) was carried on at each place. Further simplifications were introduced later, *e.g.* discontinuing the experiment at Harper-Adams College in 1927 and dropping the King Edward variety on account of certain difficulties in rogueing. It will be sufficient there-

fore for the present purpose to limit the account so as to deal only with the results obtained for the Great Scot and Kerr's Pink varieties at Rothamsted and Wye.

Rothamsted.

1925. The seed of Great Scot saved from the previous year was unfortunately lost through the depredations of rats, so that only fresh Scotch seed of this variety was planted in 1925. The types of Kerr's Pink seed planted were S, N_1, N_2, and N_3, the notation being as before. In addition to the plots which were rogued and which were reserved for seed for the following year, a twelve-fold replication of plots for weighing was laid out for the Kerr's Pink variety. The percentage of diseased plants and the yields for the various seed types are given in Table XVII.

Table XVII.

Giving percentage of virus disease present and yields per plot for four types of Kerr's Pink seed.

Seed type	No. of plots	No. of plants	% diseased	Av. wt. per plot (lb.)
S	12	238	0·4	$32{\cdot}3 \pm 1{\cdot}03$
N_1	12	231	3	$31{\cdot}3 \pm 1{\cdot}01$
N_2	12	233	14	$27{\cdot}0 \pm 0{\cdot}89$
N_3	12	232	12	$29{\cdot}8 \pm 0{\cdot}94$

In this series, deterioration is definite only in the case of the N_2 stock. It is also indicated in the N_3 stock but it has not reached significant dimensions. The general correlation of fall in yield with prevalence of virus disease is well shown in the above table.

1926. The only seed types carried on were N_1 for Great Scot and N_4 (*i.e.* the progeny of N_3) for Kerr's Pink. No weight tests were attempted, but a small lot of seed was planted, with a spacing of 2 yards, in a single row in a turnip field. No obvious disease of any sort appeared in the one-year grown Great Scot seed. With the four-year grown Kerr's Pink, virus disease of the streak type[1] was shown in the course of the season in fully 50 per cent. of the plants. No diseased plants were removed, but the course of the disease was observed by fortnightly inspections throughout the growing season. In the following year seed was taken only from those plants which showed no signs of virus disease right up to the end of the growing period. The produce of the individual plants was weighed, giving the following average yields:

[1] The relative prominence of necrotic spotting and streaking on the veins, of corrugation and mottling of the leaflets varied greatly from plant to plant, as detailed fortnightly inspections showed, so that it is probable that a very mixed type of infection was present.

Healthy. 22 plants. Av. yield per plant, 4·20 lb.

Diseased (in some cases only very slightly). 25 plants. Av. yield per plant, 2·41 lb.

1927. Again no weight tests were attempted, but two rows of plants of the two varieties (N_2 seed of Great Scot and N_5 of Kerr's Pink) were grown with swedes along one side and with fresh Scotch seed along the other. The plants were spaced at 1 yard intervals along the rows.

No definite symptoms of disease appeared in the Great Scot plants. On the other hand, the plants of Kerr's Pink, though derived from those which appeared quite free from disease in the preceding year, contained a high percentage of infected plants. Some of the latter only showed faint symptoms, but nevertheless all plants showing symptoms of disease, however slight, were rogued out early in the season (seven weeks after the date of planting). The plants so rogued out constituted 64 per cent. of the whole crop. Seed was collected from the remainder at the end of the season.

1928. A weight-test series was laid out in which a comparison was made between fresh Scotch seed and three-year grown English seed for the Great Scot variety, and between fresh Scotch seed and six-year grown English seed for the Kerr's Pink variety. During the growing season the amount of virus disease present was as follows: slightly less than 4 per cent. (mosaic type only) in Kerr's Pink and 4 to 5 per cent. (chiefly leaf-roll) in Great Scot.

The yields are given in Table XVIII.

Table XVIII.

Yields (with probable errors) of two types of seed of Great Scot and Kerr's Pink varieties.

Variety	Seed type	No. of plots	No. of plants	Av. wt. per plot (lb.)
Great Scot	S	16	300	$28·2 \pm 0·63$
,,	N_3	16	298	$27·5 \pm 0·47$
Kerr's Pink	S	14	263	$26·7 \pm 0·77$
,,	N_6	14	258	$32·2 \pm 0·68$

The difference between the yields of the two types of Great Scot seed is negligible. On the other hand, the yield of the English-grown Kerr's Pink seed is significantly greater than that of the Scotch seed.

That the English-grown seed of Kerr's Pink yielded more heavily than fresh Scotch seed is probably to be explained, as suggested earlier (p. 8), by the fact that the former were, from the conditions of the experiment, better sprouted at the time of planting. In any case it is clear that the

English-grown seed, after the drastic and apparently successful rogueing of the year before, has yielded fully as heavily as freshly imported Scotch seed.

Wye.

1925. In addition to plots for seed production for the following year, a series of weight-test plots was laid out with the following seed types—S, N_1, N_2 and N_3—for both Great Scot and Kerr's Pink varieties. No exact determination of the percentage of virus disease present could be made as the plots, which were on newly broken up famished grass land, grew poorly and showed obvious symptoms of potato hunger. The effects of the latter are not easily distinguished from certain types of virus disease. There was no doubt, however, that considerable virus disease was present, especially in the N_2 and N_3 plots. The weighings showed that the two types of seed just mentioned were definitely deteriorated, whereas the N_1 type yielded much the same as the fresh Scotch seed. The general crop yield was poor, for the reason stated, being at the rate of about 3 to 4 tons per acre, so that the comparisons obtained are of little value.

1926 and 1927. In both of these years the procedure was much the same as at Rothamsted—*i.e.* the oldest stocks (N_3 of season 1925) were carried on, with rogueing, for two years longer. The percentages of diseased plants removed were 6 and 10 per cent. in 1926 and 0 and 30 per cent. in 1927 for Great Scot and Kerr's Pink respectively. The rogueing was not carried out so drastically as at Rothamsted, and a number of plants which probably showed slight symptoms of disease were allowed to remain in the case of both varieties.

1928. A weight-test series was laid out in which fresh Scotch seed and six-year grown English seed (N_6) were compared for both varieties. The extent of the test was limited owing to the fact that some of the seed was killed by the severe frost of December 1927. During the growing season the prevalence of virus disease was found to be 10 per cent.

Table XIX.

Yields (with probable errors) of two types of seed of Great Scot and Kerr's Pink varieties.

Variety	Seed type	No. of plots	No. of plants	Av. wt. per plot (lb.)
Great Scot	S	4	80	$33 \cdot 6 \pm 0 \cdot 35$
,,	N_6	4	80	$33 \cdot 0 \pm 0 \cdot 96$
Kerr's Pink	S	4	80	$29 \cdot 4 \pm 0 \cdot 6$
,,	N_6	4	80	$28 \cdot 8 \pm 0 \cdot 3$

(nearly all leaf-roll) in Great Scot, and 28 per cent. (streak, slight in the majority of cases) in Kerr's Pink. The yields are given in Table XIX.

From this table it is seen that a slight drop in yield is shown by the English-grown seed of both varieties, but in both cases it is not significant in amount. This result may appear surprising, especially with the Kerr's Pink variety, the English-grown plots of which had a considerable percentage of virus disease, but it is to be noted that the great majority of the affected plants only showed incipient symptoms of disease, so that the actual effect on yield in the current year was negligible.

Discussion of results.

The results set out in the foregoing are best considered in two sections, referring to the periods 1922–4 and 1925–8 respectively. The work of the former period will be dealt with first.

It is clear from a study of the data presented in the various tables that there is a general parallelism between the amount of virus disease showing in any plot and the yield of that plot. Thus in the experiment at Rothamsted in 1924 there was comparatively little virus disease showing and relatively little evidence of deterioration in yielding power. On the other hand, at Wye the incidence of virus disease was much greater and there was a more striking deterioration of the English-grown stocks. This is well shown by the following comparison. Out of fifteen types of English-grown seed at Wye, thirteen showed definite deterioration. At Rothamsted, out of eight types of English-grown seed for which statistical treatment of the results could be attempted, definite deterioration was found in one case only. Then again, at each place differences in degree of attack and of deterioration appeared as among the three potato varieties used. Thus at Rothamsted the plots of Great Scot showed very slight infection with virus disease (at most 2 per cent.), and the results obtained from the weight test gave no suggestion of deterioration. The Kerr's Pink and King Edward varieties, on the other hand, showed a greater prevalence of virus disease and at the same time definite indications of deterioration, though only in one case was the fall in yield of the English-grown seed types statistically significant.

With regard to the screening experiment, the most important results were those obtained at Wye. From these it appeared that the screened plots had deteriorated to a degree quite comparable with the plots of English-grown seed grown in the open in the normal manner. There was no indication that protection of the plants from high temperatures had in any way helped them to maintain their cropping vigour. The similar

experiment at Rothamsted, though conducted on too small a scale to allow of statistical treatment of the results, nevertheless gave no colour to the suggestion that shading of potato plants had any effect on the cropping power of the resultant tubers.

The general conclusions to be drawn from the comparisons between the cropping power of early-lifted and late-lifted seed are fairly clear, and are in agreement with the view which is generally accepted nowadays and which has been put forward, for example, by Murphy and McKay (*l.c.*) and by Botjes[1]. In the experiments at Wye in 1924 the early-lifted seed definitely yielded more heavily than material of similar age in England which was lifted late. This is an illustration of the case where early lifting in the presence of a fair amount of infection with virus disease confers a distinct advantage. Nevertheless, it should be noted that it was only the *very* early-lifted seed which gave this result in a definite manner. It would seem that, in order to obtain a benefit from early lifting when virus disease is present, it might be necessary to lift so early that any advantage would be obliterated by the small yield of tubers available as seed. Again, the plots from very early-lifted seed themselves show definite indications of deterioration when compared with those from fresh Scotch seed, so that it is clear that even very early lifting, while it may retard the onset of deterioration, will not prevent it when virus disease has begun to spread to an appreciable extent.

In the corresponding tests at Rothamsted, data on the effect of early lifting which can be treated statistically are available for the two varieties Great Scot and Kerr's Pink. Very little virus disease was present in the plots of Great Scot, and all these, whether derived from early-lifted, late-lifted or Scotch seed, showed no significant differences in average yield. In fact several of the English-grown types of seed yielded more heavily than the fresh Scotch seed. For Kerr's Pink, which showed a greater prevalence of virus disease, the early-lifted seed gave a heavier yield than the late-lifted, and was fully equal to fresh Scotch seed. The condition of the plots of Kerr's Pink as regards the progress of deterioration is thus seen to be intermediate between that of the Great Scot plots at Rothamsted and the plots of all three varieties at Wye.

The general parallelism between the amount of virus disease shown and the diminution in cropping power does not appear to hold entirely in some cases. For example, in Tables V and VI it is seen that the early-lifted seed yields more heavily than the late-lifted, with no corresponding differences in the percentages of diseased plants. It is to be remembered,

[1] *Report Internat. Conf. Phytopath. and Econ. Entom., Holland,* 1923, p. 146.

however, that the data of percentage of disease present are of necessity somewhat rough, inasmuch as a mere numerical count does not represent adequately the *quantitative* effect of disease on the growth of the plant. An exact correspondence would not therefore be expected.

On the question as to whether a certain degree of immaturity in the seed may confer some advantage, even in the absence of virus disease, the above experiments give no clear indication. The data given in Tables IV and VI might be interpreted as suggesting that the beneficial effect of early lifting cannot be entirely explained on the disease theory. In one case (Table IV) the beneficial effect is very slight, in the other (Table VI) it may be largely explained in the manner indicated in the preceding paragraph. If such an effect really exists, it seems clear that it is inconsiderable, and that very careful experimentation, with virus disease excluded, would be necessary for its demonstration. It is evident, therefore, that while a purely physiological effect on yield due to early lifting has not been disproved by these experiments, it must, if it exists at all, be very small and quite subsidiary to the effects of virus disease.

The experiments of the second period (1925–8) show that, by a course of rather drastic rogueing, it is possible to maintain seed which, after six years' growth in England, gives a yield as good as that of freshly imported Scotch seed. This is a further argument against the physiological theory of deterioration. While thus it has been demonstrated that the vigour of Scotch seed when grown in England can be maintained by rogueing, it does not follow that the method is economically practicable. Very drastic, uneconomic rogueing was necessary in some cases. On the other hand, such excessive rogueing might not have been required in the later years of the test if better facilities had been available throughout, and especially in the earlier years, for the removal of the diseased plants on the first appearance of disease symptoms. The experiment was thus not a fair test of the practicability of rogueing. At the same time, even with the severe rogueing adopted, there is no anticipation that virus disease has been eliminated from any of the seed lots. There are indications that leaf-roll has been eliminated in one case, but it is certain that, short of a process of very rigorous segregation and selection, the elimination of the streak type of disease is impossible. The restoration in this way of the cropping power of a more or less infected stock of potatoes in England shows little likelihood of success as a practical proposition. A more practical line to follow would be to determine whether, by an amount of rogueing which a farmer could be expected to carry out, the life in

England of Scotch seed of good quality could be prolonged from the two to three years which is usual, to say five or six years.

SUMMARY.

1. An experiment with three varieties of potatoes (Great Scot, Kerr's Pink and King Edward) was carried out during the period 1922–24 in order to test the physiological theory of potato deterioration by determining whether the cropping power was influenced by early lifting or affected by shading the growing plants and so protecting them from overheating. The main results of this experiment were as follows:

(*a*) In the absence or very limited presence of virus disease, neither early lifting nor shading produced any significant effect on crop yield. All the types of seed (one-year grown, two-year grown, early-lifted, etc.) gave yields which were not significantly different from that of fresh Scotch seed.

(*b*) When considerable infestation with virus disease was shown, all the types of English-grown seed showed a significant falling off in yield as compared with Scotch seed. In such cases the falling off in yield was not so great with seed which had been lifted at a very early date. Thus, with virus disease present, an advantage may be gained by early lifting of the seed.

(*c*) In one case, evidence was obtained that very immature seed is intrinsically inferior in cropping power to seed lifted at the normal time, *i.e.* apart from the effects due to the unequal distribution of virus disease.

(*d*) In two cases the greater cropping power of one-year grown English seed as compared with fresh Scotch seed was demonstrated. This difference is probably to be explained on the ground of the unequal sprouting at the time of planting.

(*e*) A general correlation was observable between the amount of virus disease present in the various plots and the falling off in cropping vigour.

(*f*) No effect of a purely physiological nature arising from early lifting or from shading was indicated. Though such an effect could not be disproved, it was clear that it was of minor account and quite subsidiary to the effects of virus disease.

2. An experiment was carried out over the period 1925–8 to determine whether, by a process of rogueing, the cropping vigour of a deteriorated stock could be restored. The weight tests carried out in 1928 showed that certain English-grown stocks, some of which were in their seventh year from Scotland, yielded as heavily as Scotch seed.

3. The experiments were carried out in such a way that the statistical significance of the data obtained could be assessed. In thirty-three such experiments, where the yield of English-grown types of seed was compared with that of Scotch seed, the percentage drop in cropping power which was significant varied from 7·0 to 20·0. Over the whole series, the average figure was 11·8 per cent., corresponding to a falling off in yield of about 1·2 tons per acre.

In conclusion we wish to record our thanks, for facilities offered for this work, to the Director of the Rothamsted Experimental Station and to the Principal of the South-Eastern Agricultural College, Wye, and the Principal of the Harper-Adams Agricultural College, Newport, Salop.

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ON THE CURLY TOP DISEASE OF THE SUGAR BEET: A BIOCHEMICAL AND HISTOLOGICAL STUDY

SUMMARY OF RESULTS

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(With Plates I and II.)

The losses inflicted by the curly top disease, which is transmitted by the beet leafhopper, *Eutettix tenellus* (Bak.), are believed to be greater than those caused by any other disease of the sugar beet; instances are known in which large factories have been dismantled and removed from districts in which the leafhopper abounds. The presence of this disease threatens the entire industry in Western North America.

Various attempts have been made—dusting, natural enemies, and changing the time of planting—either to control the leafhopper or to avoid its attacks. In recent years attention has been directed particularly to the development of resistant strains. This resistance, however, seems to concern the disease itself, rather than the insect that transmits it. Sugar beets resistant to the leafhopper itself have not yet been found.

The studies summarised herein were undertaken with a view to determining the nature of the plant's resistance—chemical, physico-chemical and morphological.

Since *healthy* pumpkins and squashes have been found to be unfavourable host plants as far as the leafhopper is concerned, and since the longevity of the adult leafhopper is prolonged on *diseased* plants —since, moreover, the beets seem to become less infected as they grow older, the *younger* beets appearing to be more susceptible, not only to the leafhopper attack, but also to the disease that it transmits—special attention was paid to the properties of the younger as compared with the older leaves, and the diseased as compared with the healthy plants. Two pedigree strains of sugar beet were used, the one (51/25—3/23—P 19) markedly resistant and the other (Tracy 2769—24) markedly susceptible to the disease. The plants were grown in new 9-inch pots filled with soil of uniform composition. The experimental plots, where the observations

were made, were situated nearly 150 miles apart—at Spreckels (near Salinas, California), Berkeley (near San Francisco), and later at Davis (near Sacramento).

Physico-chemical measurements of the sap showed that the refractive index was invariably greater in the resistant strain; and, with the exception of a slight decrease in the petioles of both strains, it tended to increase with the disease. The freezing-point depression was greater in the young leaves and roots of the susceptible strain than in those of the resistant strain and tended, except in the case of the old leaves and roots of the resistant strain and the petioles of both strains, to increase with the disease. Except in the roots of diseased plants, the specific resistance was invariably higher in the susceptible strain; and, except in the roots of the susceptible strain, it increased with the disease. The increase of the refractive index, specific resistance and also mainly of freezing-point depression that accompanied the disease in the leaves of both strains indicates that the disease causes an increase in the concentration of total solids and non-electrolytes. Such an increase is markedly greater in the leaves of the susceptible compared with the resistant strain. The decrease in the specific resistance in the roots of the susceptible strain is due to the greater concentration of electrolytes and is in keeping with the fact that a morphological examination of the roots of diseased plants of this strain revealed the presence of a greater quantity of calcium oxalate.

In general, the results suggest that the sap from the resistant beets is less concentrated in total solids and non-electrolytes, and more concentrated in electrolytes, than the sap from the susceptible beets.

The differences observed in *p*H were too small to warrant any reliable conclusions. Nevertheless, with only one exception, the figures point to a slight but general *tendency* towards greater[1] acidity (*i.e.* a lower *p*H) in the leaves (especially *young* leaves) of the resistant as compared with the susceptible strain. In view of Comes' (2) suggestion that acidity is the plant's defence against its insect enemies, such a result is of special interest.

A comparison of the nitrogen content of the sap (expressed as gms. per 100 gms. of wet weight) in the susceptible and resistant strains of both healthy and diseased plants showed that the amount of nitrogen in the sap of plants of the susceptible strain was greater, both in the young leaves and in the roots, than in the corresponding portions of healthy

[1] The roots of plants of the resistant strain, on the other hand, showed a slight but general *tendency* towards lesser acidity (higher *p*H) than the corresponding parts of the susceptible beets.

and diseased plants of the resistant strain. In both strains this nitrogen content was increased markedly by the disease. In healthy plants, the younger leaves contained considerably more nitrogen than the older leaves.

Table I.

pH measurements of the sap.

	Susceptible strain				Resistant strain			
	Healthy		Diseased		Healthy		Diseased	
	Readings	Mean	Readings	Mean	Readings	Mean	Readings	Mean
Young leaves	6·012 6·011	6·01	5·968 5·979	5·97	5·960 5·960	5·96	5·786 5·782	5·78
Old leaves	6·228 6·221	6·22	6·269 6·255	6·26	6·283 6·294	6·29	6·155 6·163	6·16

Table II.

Nitrogen content of the sap.

	Susceptible strain				Resistant strain			
	Healthy		Diseased		Healthy		Diseased	
	Readings	Mean	Readings	Mean	Readings	Mean	Readings	Mean
Young leaves	·1953 ·1953	·1953	·2450 ·2460	·2455	·1471 ·1488	·1480	·1580 ·1592	·1586
Old leaves	·0885 ·0899	·0892	·0924 ·0913	·0919	·0926 ·0926	·0926	·0646 ·0648	·0647
Petioles from old leaves	·0626 ·0624	·0625	·0819 ·0804	·0812	·0704 ·0711	·0708	·0662 ·0643	·0653
Roots	·1461 ·1459	·1460	·2490 ·2502	·2496	·1271 ·1332	·1302	·1361 ·1390	·1376

The Peter Greiss calorimetric method[1] failed to reveal the presence of nitrites, as were recorded by Boncquet and Boncquet (1).

As regards *chemical analysis*, objection may be taken to almost any basis of calculation, whether it be fresh weight, dry weight, sugar-free residue or "residual dry weight" as suggested by Mason and Maskell (4). Since the sugar-free residue is probably the least fluctuating basis for calculating percentages, the comparisons here summarised are based largely upon data so calculated. It is noticeable that conclusions drawn from the physico-chemical measurements receive additional support from the chemical analyses.

As regards the leaves of healthy plants, the susceptible strain showed considerably higher figures for reducing sugars, sucrose and alcohol

[1] As described by Treadwell (6).

soluble solids, and lower figures for starch; the nitrogen content and the dry weight being practically the same.

In the susceptible strain, the disease tended to increase the amount of reducing sugars, sucrose, alcohol soluble solids and dry weight. Even healthy leaves of the susceptible strain contained nearly twice as much reducing sugars as those of the resistant strain. Mildly[1] diseased leaves of the susceptible strain contained nearly three times as much reducing sugar as the diseased leaves, with which they were comparable, of the resistant strain; severely diseased leaves contained nearly three times as much sucrose. The effect of the disease on the leaves of plants of the resistant strain was to decrease the amount of reducing sugars, sucrose, starch, alcohol soluble solids and dry weight, and to increase slightly the amount of nitrogen.

The alcohol insoluble residue[2] (fresh weight basis) increased with the disease both in the susceptible strain, and also, to a slight extent, in the resistant strain.

As regards the roots of healthy plants, the susceptible strain exhibits higher figures for sucrose and alcohol soluble solids; higher figures are also shown for starch and dry weight, but the figures for nitrogen and alcohol insoluble residue, when expressed as percentages of fresh weight, are lower; the quantity of reducing sugars is about the same.

The effect of the disease on the roots in the susceptible strain was to decrease the sucrose, dry weight and alcohol soluble solids, to increase the alcohol insoluble residue (calculated on a basis of fresh weight) and to increase the nitrogen (calculated on a basis of sugar-free residue). As regards starch, a mild attack of the disease resulted in a very slight increase, a severe attack of the disease in a slight decrease, when calculated on a basis of sugar-free residue, though a progressive increase was noticeable when calculated on a basis of fresh weight.

In the resistant strain, the effect of the disease on the roots was to decrease the amount of sucrose, dry weight and alcohol soluble solids and

[1] For analytical purposes, the samples taken from the diseased plants of the susceptible strain were divided into two lots, the one containing only severely diseased and the other only mildly diseased plants. Such a division was not possible in the case of diseased plants of the resistant strain. In considering the chemical analyses, it is important to remember that the symptoms of the disease exhibited by the plants of the resistant strain were more nearly comparable with those of the mildly diseased than with those of the severely diseased plants of the susceptible strain.

[2] It is not possible to differentiate distinctly between alcohol *soluble* solids and alcohol *insoluble* residue, since even small differences in pH might convert the one into the other and *vice versa.*

to increase slightly the amount of starch and nitrogen, the amount of reducing sugars remaining unchanged.

A fundamental difference in the two strains is shown, therefore, by the fact that in the susceptible strain the disease increases the quantity of sugars in the leaves, reducing the quantity in the roots—the loss in the one case possibly accounting for the gain in the other; whereas in the resistant strain the disease decreases the quantity of sugars in both leaves and roots. Such an accumulation of sugars in the exposed parts of the plant would doubtless render the former a more favourable host to the leafhopper than the latter. The facts suggest that the non-occurrence of severe disease in plants of the resistant strain may possibly be connected with the lessening of sugars which, in this strain, accompanies the onset of the disease. It may be that the plant becomes a less "favourable" host. Further experimental work would be required before this could be shown to be the case. Why the sugars increase in the one case and decrease in the other is a matter for further enquiry.

The leafhopper may also require and therefore be attracted to the alcohol soluble solids. These are in greater concentration in the susceptible than in the resistant strain, both in the leaves and in the roots of healthy and diseased plants; in the leaves of the susceptible strain they increase with a severe attack of the disease. It may be noted that the measurements correspond with those of the nitrogen content of the sap, which is due mainly to alcohol soluble compounds.

A further point of interest arose from a detailed consideration of the effect of the disease upon the plant.

In considering the physico-chemical measurements it has already been noted that the slight but general *tendency* towards a lower pH (*i.e.* greater acidity) in the leaves of the resistant as compared with the susceptible strain, both in the healthy and diseased plants, was in harmony with Comes' suggestion that acidity is the plant's defence against its insect enemies. The results of the chemical analyses, revealing (1) an excess of reducing sugars in the leaves of the susceptible strain, and (2) a definite decrease of reducing sugars associated with the disease in the leaves of the resistant strain, lend some further support to Comes' contention. For, according to Comes, as the quantity of reducing sugars increases in vegetable tissues, there is a corresponding decrease in acidity. The excess in the former, and the decrease in the latter case, would therefore be associated with a higher and a lower pH respectively. This appeared to be the case as regards young leaves of the susceptible, compared with the resistant strain, and the effect of disease on leaves of the resistant

strain. In diseased leaves of the susceptible strain, a percentage increase balanced a percentage decrease, *i.e.* pH was virtually constant. The sugar content (mild disease) also varied little.

The excessive *accumulation of sugars in the leaves*[1] of the diseased plants of the susceptible strain, as compared with the leaves from healthy plants of the same strain, may be *correlated with the phloem necrosis* revealed by a morphological examination of the leaves of the diseased beets. Phloem necrosis is characteristic of the disease and is not found in healthy plants. This breaking down of the phloem tissue may prevent the removal of photosynthetic products, which tend to accumulate in the form of reducing sugars and sucrose. Such an excessive accumulation of sugars is *not* found in the leaves of diseased plants of the resistant strain. For this reason, one would expect phloem necrosis to be less marked in diseased leaves from the resistant strain than in diseased leaves from the susceptible strain. Preliminary histological studies do not, however, support such an inference. A possible explanation of the phenomenon may, however, lie in the fact that this resistant strain is not naturally a

Table III.

Physico-chemical measurements of sap. (Mean readings.)

	Susceptible strain		Resistant strain	
	Healthy	Diseased	Healthy	Diseased
Young leaves				
Refractive index	1·3429	1·3466	1·3411	1·3428
Freezing-point depression	1·090	1·315	1·005	1·040
Specific resistance	19·4	25·5	18·9	23·6
pH	6·01	5·97	5·96	5·78
Old leaves				
Refractive index	1·3409	1·3421	1·3408	1·3412
Freezing-point depression	0·990	1·085	1·070	0·920
Specific resistance	17·9	25·2	15·9	21·1
pH	6·22	6·26	6·29	6·16
Petioles from old leaves				
Refractive index	1·3420	1·3415	1·3420	1·3412
Freezing-point depression	1·380	1·280	1·570	1·280
Specific resistance	12·9	15·4	11·3	15·1
pH	5·82	5·92	5·84	5·87
Roots				
Refractive index	1·3559	1·3558	1·3527	1·3538
Freezing-point depression	1·380	1·450	1·305	1·220
Specific resistance	52·1	47·6	43·6	59·7
pH	6·15	6·30	6·26	6·31

[1] Similar results were obtained by Johnston and Dore (3) in their studies on the relation of boron to the growth of the tomato plant.

good "sugar producer." The deficiency in sugar production may be aggravated by the disease.

Histological examination showed also that in the young leaves of healthy plants, the cuticle and epidermis are invariably thicker over both the palisade tissue and the vein, in the resistant than in the susceptible strain; with the exception of the cuticle over the palisade tissue, the same is true also of the diseased plants. In the old leaves of healthy plants,

Table IV.

Analyses of sugar beets expressed as percentage of sugar-free residue.*

	Susceptible strain			Resistant strain	
	4	3	1	5	2
	Healthy	Mildly diseased	Severely diseased	Healthy	Diseased
Leaves					
†Reducing sugar	23·40	24·30	27·00	12·30	8·90
Sucrose	14·20	13·90	18·50	12·00	6·4
Starch	4·17	4·28	3·89	4·91	3·82
Nitrogen	2·82	2·41	2·31	2·81	3·30
Dry weight	169·0	170·4	174·9	166·1	144·3
Alcohol soluble solids	85·6	80·7	88·6	71·5	54·3
Residue	100	100	100	100	100
Roots					
†Reducing sugar	3·0	3·20	3·06	3·0	3·0
Sucrose	227·5	188·5	131·5	170·9	99·60
Starch	2·3	2·5	2·14	1·62	1·71
Nitrogen	0·8	0·88	1·41	1·25	1·67
Dry weight	372·4	322·9	266·9	307·1	232·0
Alcohol soluble solids	283·5	230·1	170·7	213·4	147·0
Residue	100	100	100	100	100

* Owing to variation in fresh weight, dry weight, sugar content, etc., it seemed desirable to refer the results to some less fluctuating basis, such as the sugar-free residue. When referred to this basis some of the figures will necessarily be over 100 per cent.

† Expressed as glucose.

both the cuticle and epidermis over the vein are again markedly thicker in the resistant than in the susceptible strain, and the same tendency is shown in diseased plants, with the single exception of the epidermis over the palisade tissue. The difference in the thickness of cuticle and epidermis is most marked in the case of the epidermis over the vein. This was the case in both young and old leaves, whether healthy or diseased. *The tendency to a thicker epidermis and cuticle in the resistant strain may indicate some external protection from insect attack* (*epiphylaxis*).

MUMFORD.—On the Curly Top Disease of the Sugar Beet (pp. 28–35).

MUMFORD.—On the Curly Top Disease of the Sugar Beet (pp. 28–35).

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EXPLANATION OF PLATES I AND II

PLATE I.

Resistant (*R* 22) and susceptible (*S* 23) beets showing effects of mild attack of curly top. Plants selected on April 20, as *the least badly attacked of both strains* (original).

PLATE II.

Resistant (*R* 20) and susceptible (*S* 13) beets showing effects of curly-top. Plants selected on April 20, as *the most badly attacked of both strains* (original).

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APHIS AS A POSSIBLE VECTOR OF "BREAKING" IN TULIP SPECIES

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(With Plate III.)

Introduction.

Experiments on the cause of the phenomenon known as "breaking" in tulips have been carried out by Dr E. J. Collins at the John Innes Horticultural Institution from 1925 onwards, and it was because his results indicated that aphis was the probable natural carrier of a tulip "breaking virus," that the present more detailed experiments were carried out. Miss D. M. Cayley's experiments with "grafting" and "plugging" of tulip bulbs with portions of living "broken" bulbs, carried out at the Institution in 1927–8, prove that "breaking" is transmissible and are evidence that it is caused by a virus, such as induces variegation and mosaics in other plants (1). To complete the demonstration it is necessary to find the vector in nature.

The results obtained this year must necessarily be accepted with caution, and the experiments recorded below are preliminary to further and more detailed work on the subject.

Four species of aphides are known to occur on tulips, namely, *Anuraphis tulipae* B. de Fonsc., *Rhopalosiphoninus tulipaella* Theo., *Macrosiphum gei* Koch, and *Myzus persicae* Sulz.

General description.

Anuraphis tulipae, first recorded in 1841 by Boyer de Fonsecolombe, is mostly a bulb feeder, and is usually found on stored bulbs. It will, however, feed on the foliage of tulip, but later migrates down to the bulb, where it feeds beneath the dried outside scale.

One may therefore say that with this species the bulb is the normal food and that feeding on the foliage is a less usual habit. According to Davidson, evidence available seems to indicate that it is a migrating form of the usual aphidine type—the tulip being one of the intermediate food

plants (2). Other food plants of *A. tulipae* are *Crocus* sp., *Chionodoza*, *Gladiolus*, *Lilium* spp., *Scilla* sp., *Iris* spp., carrot roots and parsley roots (7).

Rhopalosiphoninus tulipaella was described by Theobald in 1916. It feeds on the growing leaves of tulip and also on the bulbs. Dr Davidson describes it as being more of a foliage feeder than has been found to be the case in the present experiment, as this species was found on bulbs bought in the ordinary way for planting in the autumn, many of which were very heavily infested (2). Colonies increased on the bulbs with a rapidity quite equal to the increase of a colony on the foliage. It can therefore be said that the normal food of this species was both bulb and foliage. Other food plants of *Rh. tulipaella* are *Viola* spp. (7)

Macrosiphum gei (*M. solanifolii* Ashmead) was described by Koch in 1855.

According to certain investigators *M. gei* carries one of the forms of potato mosaic (5). It feeds on the foliage and flower of tulip, but there is no evidence that it feeds on the bulbs. It is a pest on potatoes, and overwinters on the rose or possibly on the potato (7). The species is widely polyphagous, the other food plants besides tulips being *Spiraea ulmaria*, *S. filipendula*, *Stellaria graminea*, *Geum urbanum*, *Epilobium montanum* and *E. angustifolium* Rose, *Iris* sp., *Gladiolus* sp., *Tomato*, *Brassica rapa*, *Pyrus malus*, *Pisum sativum*, *Aster* sp., *Lactuca* spp., *Cineraria* sp., *Sonchus oleraceus*, *Tropaeolum*, *Borago*, *Lilium*, *Veronica*, *Marigold*, etc., etc. (7)

Myzus persicae was first observed in 1761 by Sulzer, but it was not until 1776 that he described it under the name of *Aphis persicae*. It has been variously recorded during the eighteenth, the nineteenth and the present century as *Aphis dianthi*, *rapae* and *dubia*, *A. vastator*, *Rhopalosiphum dianthi*, *Myzys malvae*, etc. The ova are laid in the axils of the buds of peach, nectarine, *Daphne*, *Brassica*, and probably many other plants, in October and November. The ova are green at first but later turn to shiny black. They hatch out under glass as early as January and February, but the stem-mothers occur more usually in March, and produce viviparous young about the time of flowering. In May and June these viviparous offspring become alate and fly off to other hosts. In October they usually produce alate return migrant broods which fly back to peach, nectarine, etc. Under artificial conditions in greenhouses they will reproduce themselves parthenogenetically throughout the year, so that at one period both the sexuales and the viviparous forms are found occurring side by side (7). This species shows great variation in colour;

on the tulip the young often have a definite pink shade, whereas on the radish they are as a rule lightish green in colour. The adults themselves also show varying coloration on different food plants. The following potato diseases are carried by *M. persicae*: leaf-roll (6), interveinal mosaic, common mosaic, crinkle, stipple streak, spindling sprout (3). Other diseases carried are: tobacco ring spot (6), and mosaics in the following: cucumber (4), beet, swede, rape, white clover and *Solanum nigrum* (8).

The food plants of this species include peach, nectarine, plum, cherry, almond, *Aquilegia vulgaris*, *Brassica* spp., *Beta* spp., *Crocus* sp., *Citrus* spp., *Chrysanthemum* spp., *Cactus* spp., *Dianthus* sp., *Daphne* spp., *Digitalis* sp., *Euphorbia* sp., *Fuchsia* spp., *Geranium robertiainum*, *Hyacinthus*, *Iris* sp., *Myrtus*, *Menthe hirsuta*, *Nasturtium officinale*, *Narcissus* spp., *Nicotiana* spp., *Sonchus* spp., *Solanum tuberosum* and *Tulipa* spp. (7)

Detailed description of the experiment.

The bulbs used for the experiments were "breeders" of the variety Bartigon, obtained from Cambridgeshire from a stock known to be free from "breaking," and 150 bulbs were retained as controls. They were treated in the following manner: they were fumigated with nicotine to free them from aphis; they were wiped over with alcohol, sprinkled with flowers of sulphur and planted in soil in boxes, 50 in each box. The boxes were covered with a deep layer of ashes, and remained in the open during the winter. Owing to lack of space in the heated houses, the boxes containing the control bulbs when brought indoors on March 20th, 1929, were placed in an unheated house. Consequently the control bulbs flowered somewhat later than the infested bulbs. Nicotine and soft-soap spray was applied from time to time to keep the plants free from aphis. This method of growing bulbs close together in boxes proved unsatisfactory, as many of the plants went blind. Some were discarded owing to attack of *Botrytis* sp. Fifty-nine gave normal flowers, except for slight frost damage. There was no sign of "breaking."

Control. Normal, 59. "Breaks," 0. Percentage of "breaks," 0.

Anuraphis tulipae B. de Fonsc. The stock of this aphis was obtained from a bulb store in London on the roots of *Iris* sp. and later from the same firm on bulbs of a Rembrant tulip, "Anna Maria," and the old English tulip "James Wild," flamed. The aphides were placed on bulbs of Darwin tulips known to be "broken" on November 16th, 1928, and allowed to colonise the bulbs till December 7th, when they were transferred to separate unplanted bulbs of the same stock variety Bartigon, each bulb being covered with a lamp glass or glass tube with a muslin

top. At the end of a week, on December 14th, the bulbs were so badly affected with *Penicillium* spp. that they had to be removed, treated with alcohol, fumigated with nicotine, and planted in boxes. They were then plunged in the open. In one box the bulbs were planted untreated with the aphides still on them. On March 20th, 1929, the boxes were brought into the Intermediate House, temp. 65–70° F., and the bulbs grown on in the ordinary way. Nicotine and soft-soap spray was used from time to time to ensure no further infestation of the bulbs or foliage. In the earlier stages of leaf development, before flowering occurred, some of the leaves had a mottled appearance similar to that associated with "broken" tulips. This mottling was, however, fainter than is usually the case in a "broken" variety, and by the time the flowers had all developed the "broken" appearance of the leaves had disappeared. Eighteen bulbs were attacked by *Botrytis* sp. and were discarded. There was no perceptible difference between the plants from fumigated bulbs and the plants from bulbs on which the aphides had been left. The results of this experiment were negative. No "breaking" occurred.

A. tulipae. Normal, 82. "Broken," 0. Percentage of "breaks," 0.

The experiment with *Rhopalosiphoninus tulipaella* Theo. was in all respects similar to the above. The stock of aphides was obtained from bulbs bought by the Institution in November 1928. Again the results were negative.

R. tulipaella. Normal, 68. "Broken," 0. Percentage of "breaks," 0.

In the experiment with *Macrosiphum gei* Koch the stock was collected from "unbroken" tulips in the garden of the Institution in July 1928 and was reared on lettuce throughout the winter. On February 5th, 1929, the aphides were transferred to the growing shoots of tulip varieties known to be "broken." Three weeks later on February 26th the aphides were transferred to the foliage of the stock variety Bartigon, planted singly in 6 in. pots. Each bulb was covered with a lamp glass of the "hurricane" pattern and muslin was placed over the top, kept in place by an elastic band. Not less than twelve aphides were transferred to each plant. The plants were kept in a separate compartment of the intermediate house, temp. 65–70° F.

On March 12th, a fortnight after the first infestation, the glasses were removed and the plants were sprayed with nicotine and soft soap. This spraying was repeated from time to time to ensure that the plants were kept clean. When the flowers appeared three were definitely "broken" with the ordinary white streak; one flower showed very slight signs of darker streaks of red on the red self ground colour. Three flowers showed

a white "splash" marking on one of the outer petals, in addition, one had a very slight white "splash"—this might have no relation to "breaking" and be due to physiological causes. Whether the "splash" bears any relation to "breaking" or not remains to be seen when the bulbs come into flower next year.

A few bulbs affected by *Botrytis* sp. were discarded. Thirty-three plants were blind.

Macrosiphum gei. Normal, 45. *Definite* "breaks," 3; very slight red "break," 1; "splash," 3; slight "splash," 1; total, 8. Blind, 33. Percentage of *definite* "breaks," 5·6.

Myzus persicae Sulz. was received from Dr Kenneth M. Smith of the School of Agriculture, Cambridge, in the late summer of 1928. The aphides were first raised on spinach, but later on radish, as the latter was found to be a better food plant and survived longer than spinach when colonised with this aphis. On February 5th, 1929, the aphides were placed on the foliage of "broken" bulbs. Twenty-two days later, on February 27th, the aphides were transferred to the foliage of the stock variety Bartigon growing in separate pots each covered with a hurricane lamp glass with muslin top. Not less than twelve aphides were placed on each plant. A fortnight later, on March 13th, the lamp glasses were removed and the plants sprayed with nicotine and soft soap. This treatment was continued from time to time to secure immunity from further infection. The plants were kept in a separate compartment of the intermediate house, temp. 65–70° F. When the flowers appeared, five were heavily marked with dark red streaks on the red self ground colour—a definite form of "break" though no white ground showed. This form of "breaking" does occur from time to time among garden tulips in varieties which are dark crimson or purple in the breeder state. It is still uncertain whether it is distinct from the ordinary form of "breaking" in which streaks and stripes of the pure white or yellow of the mesophyll are revealed. Four flowers had both white and red streaks, and four flowers had the very marked red streaks, but also showed some white. All the above were definitely "broken." Of the remainder 39 plants gave normal unbroken flowers, and 31 were blind. This peculiar form of "breaking," *i.e.* the dark red streaks on the red ground, seems to be very closely associated with *Myzus persicae*, as in all the cases where "breaking" occurred in this experiment the red streak was invariably present even when in conjunction with the white streak. In the case of *Macrosiphum gei* only one flower showed this form of "breaking," and that was so slightly perceptible as to be doubtful.

M. persicae. Normal, 39. "Breaks": dark red streak, 5; dark red streak and little white, 4; dark red streak and white streak, 4; total 13. Blind, 31. Percentage of "breaks," 25.

So far as can be judged from the experiments—which admittedly are of a preliminary nature—*Myzus persicae* is a vector of the virus or one of the virus diseases, which are believed to cause the phenomenon of "breaking" in tulips. There is also some evidence to suggest that *Macrosiphum gei* is also a vector. It is obviously impossible at this stage to enter into any discussion as to whether or not there is more than one virus concerned, but the possibility must be kept in mind. It should be noted that in the two latter experiments with *Macrosiphum gei* and *Myzus persicae* only a short interval of six weeks intervened between infestation with aphides from "broken" tulips and the flowering. Even at the time of infestation the flower buds were already formed within the bulb, and the visible effect of the virus may be deferred until another year. The experiments are being continued and it is hoped to gain some more knowledge of the subject as the work develops.

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Summary.

1. *Myzus persicae* Sulz. is suggested as a vector of the virus of transmissible variegation known as "breaking" in tulips.

2. "Red-streak break" is associated with *Myzus persicae.*

3. *Macrosiphum gei* Koch possibly carries "break" in a lesser degree and is associated with "white streak."

4. *Anuraphis tulipae* B. de Fonse. and *Rhopalosiphoninus tulipaella* Theo. have both so far shown negative results.

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EXPLANATION OF PLATE III

PLATE III.

Figs. 1, 2. Typical "break" in the tulip variety Bartigon, after infestation by *Macrosiphum gei*.

Figs. 3, 4. Dark and red streak "break" in the tulip variety Bartigon after infestation by *Myzus persicae*. The streaks here shown are deep red on the "self" red ground colour.

(*Received July* 1*st*, 1929.)

McKENNY HUGHES.—Aphis as a Possible Vector of "Breaking" in Tulip Species (pp. 36–42).

GIBBERELLA SAUBINETII (MONT.) SACC. ON BRITISH CEREALS

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(With 2 Text-figures.)

CONTENTS

Introduction.

During the winter of 1928–9 considerable quantities of American feeding barley were imported into this country from the U.S.A. Some of this proved toxic to farm animals, especially to pigs, and when examined at the Plant Pathological Laboratory of the Ministry of Agriculture and Fisheries it was found to be infected with *Gibberella Saubinetii*. It is known, particularly from the work of Russian and other Continental investigators that cereals attacked by this fungus become toxic. The diseased grains from American sources may not have the same toxic properties as occur in affected European and Asiatic cereals, but at present there is no information on this point.

The occurrence of *G. Saubinetii* on wheat and other cereals throughout Northern Europe and Asia has been observed since 1882, the investigations being reviewed and extended by Naumov (3). It was first described in the U.S.A. by Selby (4), and has been studied in various parts of that

country since then as being the chief cause of "wheat scab." A résumé of these investigations is given by Atanasoff (1). It might be expected that the fungus would occur freely in Britain, since our climatic conditions appear to be favourable, and large quantities of grain have long been imported from these two areas. So far as the writer can ascertain, *G. Saubinetii* has not been recorded on any English cereal crop, and the present record of its occurrence on wheat and barley grains is, therefore, the first for this country. Possibly it is more common than is generally supposed, and might have escaped detection, firstly, because its perithecial stage is, apparently, rare or absent in nature under our climatic conditions; and, secondly, because its conidia are transitory and rarely abundant, and those that occur may not serve for the recognition of the fungus. The present paper, dealing with the methods of culture and the identification of *G. Saubinetii*, is published mainly for the purpose of obtaining the help of other mycologists in ascertaining the distribution and prevalence of this fungus in our home-grown cereal crops. This information may thus be included in a later, more detailed report concerning the morphology, physiology, and pathogenicity of the English strain in comparison with the Continental and American strains.

Isolation of *G. Saubinetii* from wheat grains.

In October 1928 the writer received from Mr W. A. R. Dillon Weston, of the School of Agriculture, Cambridge, eight grains of wheat taken from a sample of English-grown seed from an unrecorded district. Seven of these grains showed a reddish discoloration mainly about the germ end; amongst the apical hairs "black moulds" (Cladosporium, Alternaria, Epicoccum and Fumago) were present. After external disinfection[1] these grains were kept in sterile moist chambers at room temperature ranging from 8° to 16° C. At the end of 16 days three grains were overrun with "black moulds," whilst five others bore a dense white mycelium beneath which the coat of the grain was of carmine colour. From this mycelium conidia were obtained which proved to belong to *Fusarium culmorum*, *F. avenaceum*, and *F. arthrosporioides*. Further, two of the grains bore conidia of a single type and extremely few in number, and similar conidia were seen occasionally amongst those previously mentioned. It was thought that these slightly different conidia might belong to *F. culmorum* var. *leteius* Sherb., and, as the writer had stated (2) that this particular variety had not been found on cereals in this country, special attention

[1] 1 per cent. formaldehyde solution for 1 minute after preliminary steeping, then rinsed well in sterile water.

was devoted to them. Single conidia were used as sources of cultures, but, owing to the difficulty of recognising these particular conidia with certainty, many cultures turned out to be *F. culmorum*. More than a dozen isolations, however, yielded a species not immediately identified, but which proved eventually to be *G. Saubinetii*. The original infected grains were kept under observation for a further period, the dishes being kept moist, and being submitted during alternate weeks to temperatures of 20°–21° C. and 10°–15° C. respectively. After about a month under these conditions small sporodochia were observed at a few places where the covering mycelium had previously been torn. Single-conidium cultures were secured from these sporodochia; the conidia are illustrated in Fig. 1, I. During February, *i.e.* after two months' incubation, perithecia were first observed, and by March some of these were found to contain asci and ascospores. At this time the grains were covered with a matted mycelium, beneath which was a carmine plectenchyma; this plectenchyma had, in places, become hard and blue-black where clustered perithecia occurred in patches. The perithecia, asci and ascospores resembled those shown in Fig. 2. Single-ascospore cultures prepared from this source eventually yielded growths identical in every respect with the cultures derived from single conidia. The life cycle of *G. Saubinetii* had, therefore, been completed on naturally infected wheat grains, and the occurrence of this fungus on English-grown wheat was established. This was verified in the cultural work described in the following pages.

ISOLATION OF *G. SAUBINETII* FROM BARLEY GRAINS.

During the early months of 1929 samples of barley grain were examined for the presence of *G. Saubinetii*. Further search was suspended when one sample, from a Durham-grown crop, yielded this fungus. In seeking suitable grains for examination those were selected which showed a reddish or reddish brown discoloration as a dot or patch on the husk, with apparent freedom from "black moulds." Such grains were disinfected externally and kept in sterile moist chambers. No such successful results were obtained with barley as occurred with the wheat grains; sooner or later the barley grains were overrun with various fungi, more frequently Cephalosporium, which also causes a reddish discoloration of grain. Soon after placing discoloured barley grains under moist conditions the reddish patches became brighter in colour, occasionally carmine, and here they bore a delicate hyphal growth. The method of isolating the fungus eventually adopted was to scrape at a restricted spot on one of these carmine patches with a sterile needle, and place whatever

was picked up on a set plate of malt gelatine. From any resulting growths resembling that of Fusarium small pieces from the margins were transferred to wheat-meal agar slants. Since the unwanted fungi could, as a rule, be recognised on the dishes and thus avoided, the slants were mainly Fusarium growths, and those that gave conidia more or less readily were identified and rejected. Such growths as did not yield conidia readily or at all were retained, and sub-cultures were made from mycelium close to, or, where possible actually forming, the plectenchyma on the surface of the medium. Amongst the microconidia produced on the aerial mycelium of some of these slants, there appeared occasionally a conidium resembling the macroconidium of *G. Saubinetii*. Conidia of this type were selected from different slants and single-conidium cultures prepared from them. From this set of cultures the first six proving to be *G. Saubinetii* were retained. Each of these six cultures had been obtained from a single conidium, and each condium had been derived from a different grain. The cultures have been grown on to "normal" form, their purity ensured by plating out conidia from sporodochia and comparing triplicates throughout their growth and, finally, bringing them to the mature perithecial stage. Single-ascospore cultures prepared from the latter reproduced the normal conidial stage. Its life cycle having been carried through from its mycelial stage on barley grains, the occurrence of this fungus on English-grown barley is established.

Cultural methods: General remarks.

The circuitous method practised for isolating *G. Saubinetii* from the barley grains calls for some explanatory remarks. In the first place it must be emphasised that it is impossible to diagnose a specific Fusarium disease of a cereal from a superficial examination of affected grains. The latter may show a discoloration as a reddish dot or patch, or a brownish marking, but sometimes they show no discoloration at all. The reddish discoloration, which is typical, occurs in the grains when the subcuticular plectenchyma is well developed, but the colour shade produced by *G. Saubinetii*, *F. culmorum*, *F. avenaceum* and *F. herbarum* is the same in each case, so that red grains are not characteristic of any one of these organisms. Grains affected by *G. Saubinetii* sometimes bear externally a film of mycelial tissue without conidia. Wollenweber (5) ascribes this to the fact that in this species the conidia are generally very short-lived, soon germinating and anastomosing to form a stroma, *i.e.* the film. In cultures the pionnotes formed on potato plug and some other substrata soon resolves itself into a thallus-like stroma. Thus in nature the stroma

is common, whilst conidia are comparatively rare, and conidia may not be found at all on incubated grain that is much contaminated with other organisms. Atanasoff (1) states that in order to promote the production of conidia in cultures it was necessary to contaminate the cultures purposely with a certain bacterium (unidentified), which, he found, also favoured the subsequent formation of perithecia. It is worthy of mention that in the present investigation any casual bacterial contamination has retarded or inhibited mycelial growth and production of conidia, both in artificial cultures and on infected grains.

In the second place, using the mycelial and conidial characters alone, it is frequently impossible to differentiate between the Fusarium stage of *G. Saubinetii* and *F. culmorum*. When either of these fungi is isolated from different hosts and grown in pure culture, it shows considerable variation in size and shape of conidia, although merely different isolations of the same fungus. Because of such variations the conidia of these two fungi sometimes agree more closely than would appear from illustrations of characteristic forms, and identification is correspondingly difficult. Further, conidia from the outside and mycelium from the inside of an affected grain or plant, isolated separately and grown under the same conditions, may show differences in general appearance and still be the same organism. It is clear, therefore, that isolations made as described on p. 45, or when made from conidia or aerial mycelium, as is generally done, must be grown on until definite diagnostic characters are obtained. For the two fungi in question the distinctive features are that *G. Saubinetii* produces perithecia but no chlamydospores, whilst *F. culmorum* and its related species produce chlamydospores but no perithecia.

Since in the present investigation both the conidial and perithecial stages of *G. Saubinetii* had been produced on naturally infected wheat grains, whereas neither of these stages was obtained by incubating barley grains, comparative cultural work was carried out in order to ascertain whether there were two distinct strains of the fungus on these cereals. The results proved that they were one and the same strain, the differences observed under incubation being due to other factors, mainly to the deleterious effects of other micro-organisms on the barley. Since the macroscopic and microscopic characters of the fungus obtained from the two cereals are alike a single description will suffice. Some reference will be made to variations in growth or appearance arising from the use of different kinds of inoculative material, but as these variation forms can be converted one to the other by appropriate methods, they are to be regarded as normal variations within the species.

Of the many different media used, only a few are required for purposes of identification; sugar media and potato dextrose agar, useful for many Fusarium species, are of little use for *G. Saubinetii*. The methods of preparing certain media are given below[1].

Single-conidium cultures.

The method adopted for securing single-conidium cultures has been described in a previous publication (2); in the present investigation slant cultures were taken from three of the single-conidium growths per dilution plate. From each individual marginal portions were transferred to slants of a number of different media. It was found that by keeping these slant cultures in good daylight, or sunlight if not strong, and at moderately high but varying temperature, *e.g.* in an ordinary warm room rather than at the constant temperature of an incubator, the development of the organism was more favourable for purposes of identification.

(a) *Inoculations from aerial mycelium and mycelial conidia.*

When slants are inoculated from non-sporing aerial mycelium, or from an individual arising on a plate from a single conidium from aerial mycelium, the resulting growth is usually as follows:

Wheat-meal agar. Aerial mycelium abundant, white with interspersed shades of carmine; the surface of the medium pomegranate purple to Bordeaux[2]; as a rule, no spores of any kind produced.

[1] *Wheat-meal agar.* "Whole" wheat-meal, 30 gm., stirred in 400 c.c. of cold tap water; this warmed up to 60° C. in a water bath, and kept between 60° and 70° C. for 1 hour. Filter through twofold cheese cloth. Add agar 7·5 gm., make up to 500 c.c., and steam until dissolved. Sterilise by steaming for 30 min. on 3 days.

Oat agar (hard). "Quaker" oats ground finely in a mortar, 50 gm. Prepare as wheat-meal agar, but here use 15 gm. of agar for 500 c.c. of medium.

Potato agar. Old potatoes peeled and sliced, 100 gm. Add 300 c.c. of cold tap water; cook in a water bath for 40 min. without disintegrating the potato. Filter through fourfold cheese cloth. Add agar 10 gm., make up to 500 c.c. and steam until dissolved. Sterilise at 10 lb. pressure for 20 min.

Salts-glycerine agar. $NaNO_3$ 2 gm., K_2HPO_4 1 gm., $MgSO_4$ 0·5 gm., KCl 0·5 gm., $FeSO_4$ 0·01 gm. Dissolve the salts in distilled water. Add glycerine 30 gm. and agar 15 gm. Steam until dissolved; then make up to 1000 c.c. with distilled water, the whole being at 60° C. Adjust with NaOH to pH 6·6. Sterilise at 10 lb. pressure for 20 min. Pure chemicals used.

Cooked wheat media. Cover wheat grains in wide test tubes with cold water, warm up to 60° C. and hold at this temperature for 6 to 12 hours. Pour off the unabsorbed water, plug the tubes, and sterilise by steaming for 20 min. on three successive days. The grains remain whole, or merely burst, without becoming mushy.

[2] Ridgway's *Colour Standards and Nomenclature.* The technical terms used are defined in *Journn. Agric. Res.* (6)

Oat agar. Aerial mycelium as on wheat-meal; medium changes to shades of honey yellow and ochraceous tawny, with occasional surface patches between carmine and Bordeaux; generally a few microconidia on the mycelium, 1- to 3-septate and small, but occasionally a more typical, larger, 5-septate one also.

Salts-glycerine agar and *Potato agar.* Aerial mycelium moderate, entirely white; no coloration of medium; no conidia.

On all these media, as the cultures age up to about two months, sclerotial structures are developed. They arise on the surface of the medium below the mycelial covering or as a central nucleus of a clumped mass of mycelium, having a diameter of 0·5 mm. when first observed, and frequently increasing to the size of a pea. They consist of small-celled pseudoparenchyma, are honey yellow in colour, are at first tough and later semi-brittle. Transfer of the mycelial growth to fresh slants of similar media increases the tendency to the production of white aerial mycelium and loss of coloration of the medium. If, however, transfers be made from the plectenchymatic mycelium formed on oat or wheat-meal media, the sub-culture tends to a reduction of mycelium, an increase of colour, and comparatively numerous mycelial conidia when about one month old.

(b) *Inoculations from sporodochial conidia.*

To bring the fungus to a stage where it bears sporodochia is an essential for the production of the later perithecial stage on artificial media. The first step in this process is the sub-culturing from plectenchymatic material mentioned above. In such sub-cultures sporodochia are usually present amongst the aerial mycelium, but are masked. If the mycelium be torn with a sterile wire the sporodochia will become visible later between and along the torn mycelial strands. They commence as minute droplets or films of water, then become tinted globules consisting of slimy masses of conidia, the colour ranging from pale ochraceous to salmon orange according to the extent to which carmine diffuses from the mycelium. In these sporodochia 90 per cent. of the conidia may be 5-septate and of normal shape, the others being 3-, 4-, 6-, 7-, and occasionally 8- or 9-septate (Fig. 1, II, III, IV). Large, tubercular sporodochia, such as are so common in *F. culmorum* and allied species, have not been observed. Whether sub-cultures be made by direct transfer of sporodochial conidia, or from the individuals produced by single conidia on plates, the resulting growth is usually the same, though delayed in the latter case, and is as follows:

Wheat-meal agar. Aerial mycelium abundant, white interspersed with shades of carmine; the surface of the medium strongly coloured carmine changing to Bordeaux.

By removing the aerial mycelium from along one-half of the slant when the culture is about three weeks old there will be found from the fourth or fifth week onwards a dry, ox-blood red plectenchyma bearing vast numbers of conidia. In the remaining aerial mycelium are scattered very numerous sporodochia and developing perithecia.

Oat agar. Aerial mycelium abundant, with much carmine and yellow coloration; the surface of the medium of the lower half of the slant carmine to pomegranate purple, the upper half ochraceous tawny. After stripping some of the aerial mycelium as described above, a dry plectenchyma of hazel to brick-red colour is revealed, this bearing conidia abundantly. The remaining aerial mycelium bears sporodochia and perithecia scattered singly throughout.

Salts-glycerine agar. Aerial mycelium abundant, white but with carmine and yellow shades diffused giving the whole a rosy tint; the medium assumes a rosy shade throughout. After removal of some of the aerial mycelium a faintly rose tinted plectenchyma is exposed bearing numerous minute, brownish-white sporodochia close together but not actually coalescent—what Sherbakoff terms a pseudopionnotes. The conidia of smaller general size, but rapidly swelling, germinating and anastomosing to a stroma (Fig. 1, V). After one month numerous incipient blue-black or blue-green perithecia in the pseudopionnotal layer colour the slant.

Potato agar. Aerial mycelium poorly developed, in places absent; surface of the medium of pale Bordeaux shade. On the plectenchyma is a poorly developed pseudopionnotes, of ochraceus colour; of these pionnotal conidia about 50 per cent. only are 5-septate, the remainder being 3- or 4-septate together with abnormal forms (Fig. 1, VI). The addition of 1 per cent. of dextrose to this medium favours the production of perithecia, but 5 per cent. does not do so.

(c) *Production of mature perithecia from conidial stage.*

Whilst recognisable perithecia have been produced in cultures originating from a single conidium on the above-mentioned media, they have not yet been brought to the ascospore stage. The mature stage has, however, been produced by a simple process, which appears to be suitable for the speedy and unfailing production of this perfect stage in the laboratory. Wheat grains cooked in wide test-tubes were used instead of artificial media, and inoculated as for sub-cultures from pure cultures on slants. The inoculum for the grain cultures here described was in all cases aerial mycelium from oat-agar medium. The inoculated grain was kept at room temperature (8° to 15° C.) until well covered with fungal growth. It was then transferred to sterilised soil in pots, spread in the surface soil, and kept in a greenhouse (6° to 25° C. generally, but up to 32° C. on sunny days). The pots were kept covered with Petri dish lids, and the soil was kept moist by absorption of water from a supply in pot-saucers. The grains on the surface of the soil first produced an aerial mycelium which eventually disappeared or remained as a thin stroma. After about six weeks most of these grains bore a larger or smaller crust of clustered perithecia in mature condition (Fig. 2, II). Single-ascospore

cultures were secured from perithecia derived respectively from the wheat and barley sources mentioned, and these served for comparative culture observations.

By using aqueous extract of sterilised soil instead of water for steeping and cooking the grain medium, and turning the cultures prepared on this grain into sterile Petri dishes kept moist continuously, mature perithecia were obtained in the laboratory with little or no contamination. Pure cultures from single ascospores were prepared from this source also.

Perithecia were produced on living grains during part of the work on pathogenicity which is in progress. Wheat grains were inoculated by contact with pure cultures, and others by immersion in an aqueous suspension of conidia; inoculations from the wheat and barley "strains" were kept separate. Barley-seed grain was similarly treated. The grains, along with controls, were grown on sterilised sand, under bell jars[1]. After removing the shoots for other purposes, the grains were kept in the same moist atmosphere, the temperature being between 10° and 18° C., but possibly considerably higher under the glass jars standing in the sunlight (March 1929). Towards the end of the third month of the experiment it was found that of twenty grains per dish an average of eight yielded mature perithecia. The production of an exactly similar perfect stage on 40 per cent. of the inoculated grains, and complete absence from the controls, proved that the perithecial stage was the result of the inoculation, in spite of considerable contamination found at the end of the period. It may be mentioned here that the living shoots of both wheat and barley were attacked by both "strains," this cross-inoculation thus supporting the conclusion, drawn from cultural work, that this organism is one strain only of *G. Saubinetii*.

Single-ascospore cultures.

There is little difficulty in procuring single-ascospore cultures of *G. Saubinetii*. The perithecium in water, with slight pressure on the cover glass, discharges the ripe ascospores, and mature asci which emerge soon disintegrate, leaving the ascospores free. By passing through successive drops of water those can be seen which contain three or four perfectly isolated ascospores, and the latter can be transferred to malt gelatine for plating out. Transfers from the margins of at least three single-ascospore growths per perithecium were made simultaneously to four or more different media, for comparison of single-ascospore growths from the same and from different perithecia. In no instance was any

[1] For details of the method of conducting this experiment see Lit. Ref. (2), p. 216.

variation found in the growths from single ascospores from any one perithecium. But from perithecia produced under different conditions of substratum, temperature and moisture, there may be a distinct difference in the form of growth in the first sub-culture. There appear to be two such forms, each showing its distinctive features on twelve different kinds of artificial media, but the conditions which determine the subsequent form of growth are not yet clearly understood.

The first transfers to slants from plated-out ascospores may yield abundant mycelium, coloration of media, and a few mycelial microconidia, the cultures being intermediate in form between those from mycelial and those from sporodochial inoculations on similar media (pp. 48, 49). That is, there is an immediate reproduction by the ascospore of the conidial (Fusarium) state. In the second form of growth, the first transfers to media slants from plated-out ascospores are described in more detail. The transfer in all cases was a tiny piece of malt gelatine impregnated with mycelium taken from the margin of an individual on the plate.

Wheat-meal agar. A carmine-coloured growth in the surface of the medium; as this extends upwards and downwards the central area becomes deep carmine, Bordeaux, blue-black, violet-black; the black area, circular, with diameter equal to the width of the slant, forms a hard, black crust. The crust consists of reddish-brown plectenchymatic mycelium studded with developing perithecia, the latter being blue-black in colour. The upper part of the slant is of carmine to Bordeaux colour, covered with downy, white aerial mycelium, this bearing in moderate number microconidia of the Fusarium stage.

Oat agar. A carmine-coloured growth gradually extending over the whole slant, and becoming covered with a short, woolly, dingy white aerial mycelium. Beneath this mycelium perithecia are produced on the plectenchyma but are more scattered and do not form a black crust. Mycelial conidia rather more numerous and regular in shape.

Salts-glycerine agar. A scarcely visible growth of mycelium, like a delicate web in the surface of the medium, radiating from the inoculum centre. On this mycelium perithecia are produced abundantly, close together but not forming a crust, giving the culture a blue-green, then blue-black, colour. Microconidia of the Fusarium stage present, but few in number and abnormal in shape and size; they germinate speedily and produce a white, downy aerial mycelium over the blue-black area.

Potato agar. Growth as on salts-glycerine, but no perithecia. With 1 per cent. dextrose, a powdery to very short woolly aerial growth with a few rudimentary perithecia.

Transfers of aerial mycelium (with microconidia) from any of these cultures to fresh similar media gives rise to the usual Fusarium stage of growth as obtained in cultures started from the ordinary mycelial form.

It is evident, therefore, that the ascospore stage reproduces the conidial (Fusarium) stage quite readily, either directly and clearly, or through an intermediate form in which both perithecial and conidial stages are intermingled.

SPORE MEASUREMENTS.

Measurements of conidia of G. Saubinetii *isolated from English cereals*, 1928–9.

On naturally infected wheat grains; 25 days' incubation:

3-septate, 3 %, 30–50 × 3·0–4·5μ; average, 38 × 4·2μ.
5-septate, 95 %, 48–67 × 4·2–5·5μ; average, 56 × 4·9μ.
6-septate, 1 %, 60–72·8 × 5·0–5·5μ; average, 64 × 5·2μ.
7-septate, 1 % } 73–78·5 × 5·6μ.
8-septate, very rare }

On dying shoot of seedling barley, grown under warm, moist conditions; sporodochial conidia from soft, ochraceous salmon mass:

3-septate, 5 %, 45·5–50·0 × 4·5–5·0μ; average, 48·5 × 4·9μ.
5-septate, 90 %, 50·0–65·0 × 5·0–5·9μ; average, 60·0 × 5·5μ.
6-, 7-, 8-septate occasional, 65–80 × 5·8–7·0μ.
1-, 4-septate occasional.

Pure culture on wheat-meal agar, 3 months old; sporodochial conidia:

3-septate, 12–15 %, 33·8–44·2 × 4·6–5·0μ; average, 40·5 × 4·9μ.
4-septate, 8–10 %, 33·8–49·4 × 4·9–5·3μ; average, 45·0 × 5·2μ.
5-septate, 75–80 %, 52·0–60·0 × 4·8–5·2μ; average, 58·0 × 5·1μ.
extremes 41·6–77·6 × 4·5–5·5μ.

Pure culture on potato agar, 14 days old; pseudopionnotal conidia; great variation in size of equiseptate conidia; shapes equally spindle and sickle forms; the following measurements are for the most typical average forms:

3-septate, 32 %, 34·0–50·0 × 4·0–5·2μ; average, 40·7 × 4·8μ.
4-septate, 28 %, 41·5–52·0 × 4·6–5·2μ; average, 46·0 × 5·1μ.
5-septate, 35 %, 44·2–54·5 × 4·9–5·3μ; average, 49·1 × 5·2μ.
6-, 7-, 8-, 9-, 1-septate make up the remaining number as casuals.

As the conidia rapidly swell for germination abnormal sizes are common, *e.g.* 4-septate, 60 × 6·8μ; 9-septate, 80·6 × 7·5μ.

The conidia are very similar in shape to those of *F. culmorum*, but in general are longer and more slender, have thinner walls, less prominent septa, and more prominent "foot," and lack that constriction which is commonly present towards the base in *F. culmorum*. Whilst typically 5-septate, conidia with 3 septa are common, 4 septa fairly common, 6 septa occasional, and 7, 8 and 9 septa rare. In masses the conidia are

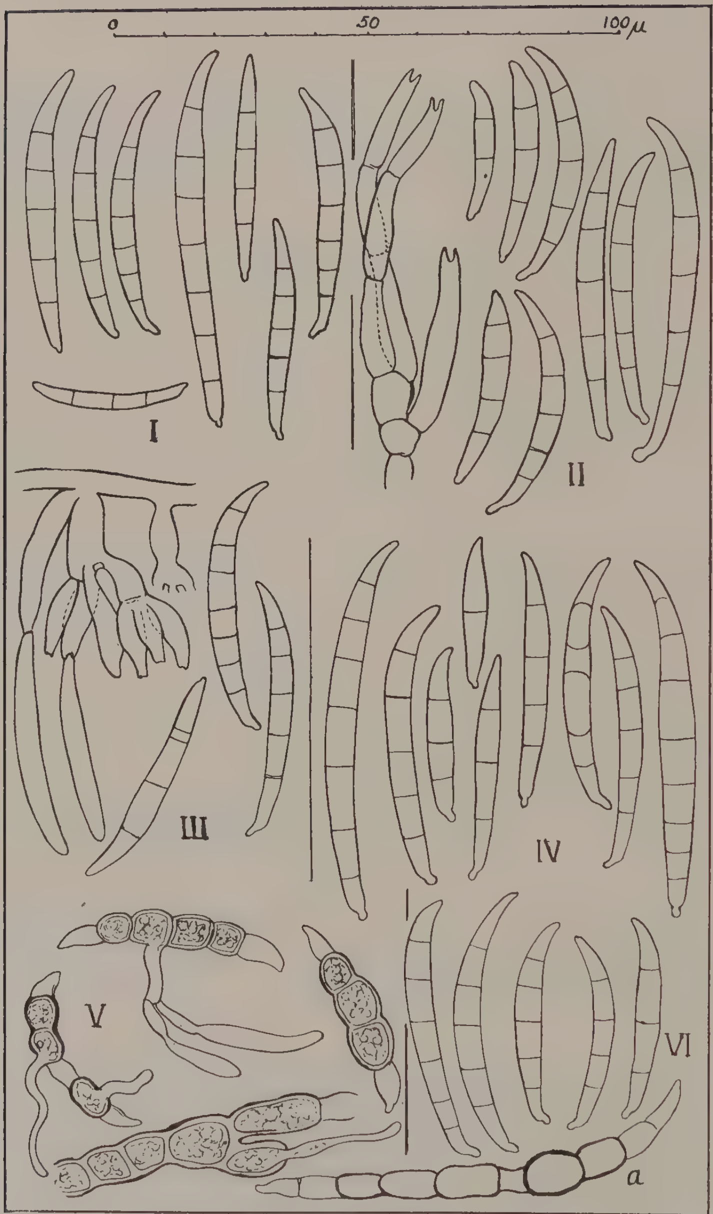

Fig. 1. *Gibberella Saubinetii* (Mont.) Sacc. Isolated from wheat and barley grains in England.

I. Conidia from naturally infected wheat grains incubated in moist Petri dishes.

II. Conidia and part of a sporodochial conidiophore from sporodochia produced under comparatively dry conditions, viz. on wheat-meal agar in a Petri dish; 3 months after transplanting.

III. Conidia and conidiophores from sporodochia on oat agar; 19 days after transfer from a single-conidium individual.

IV. Conidia from sporodochia on wheat seedling base, after artificially inoculated seed had been grown under "abnormal conditions" (p. 51).

V. Conidial and mycelial segments from salts-glycerine agar; swelling and germinating in the pseudopionnotes at 3 weeks old.

VI. Conidia from the pseudopionnotes on potato agar; (*a*) an abnormal form.

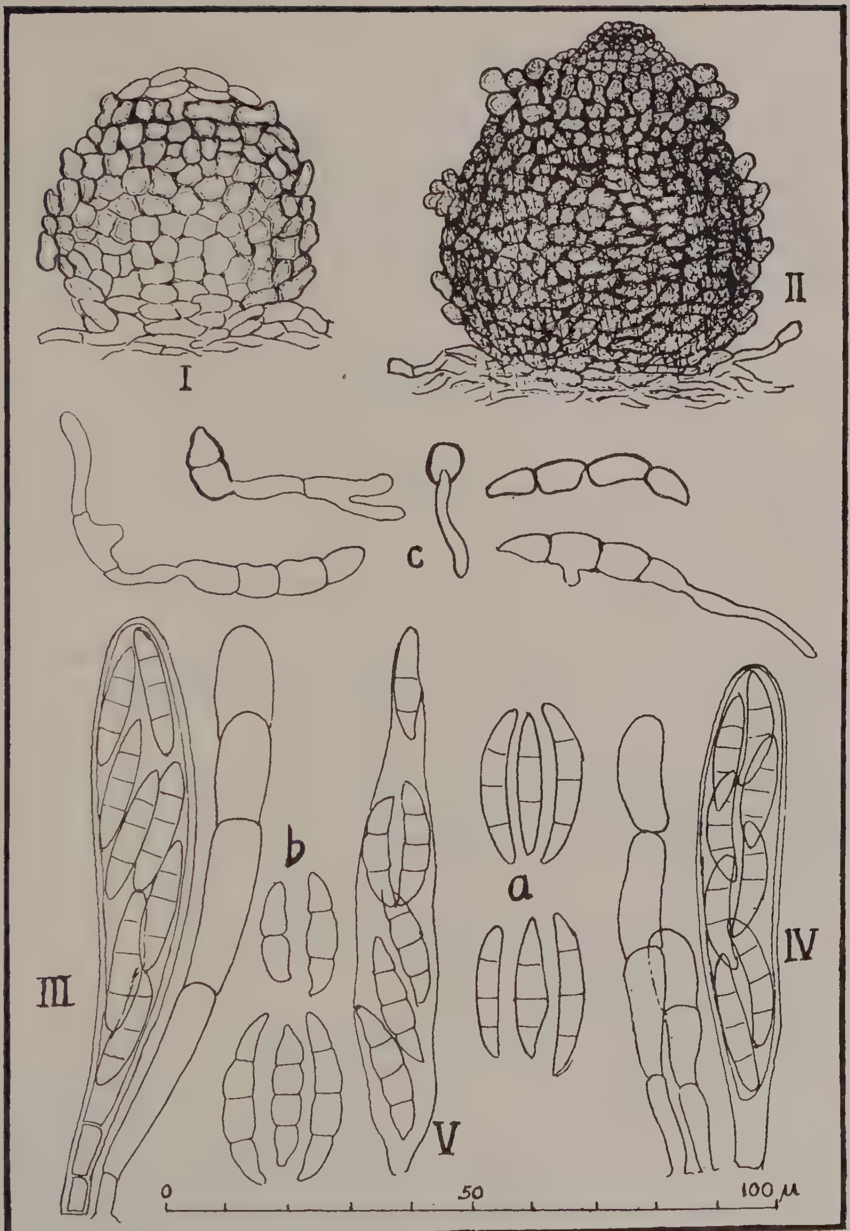

Fig. 2. *Gibberella Saubinetii* (Mont.) Sacc. Isolated from wheat grains; an identical form also isolated from barley grains.

I. Immature perithecium; beak with thin, almost colourless walls above a collar of thick-walled cells; actual size 0·075 mm. (semi-diagrammatic).

II. Mature perithecium from a growing grain of wheat; verrucose type, with protuberant cells at the collar and elsewhere; smooth-walled perithecia equally common; actual size 0·25 mm.

III. Ascus and paraphysis, approaching maturity; produced under moist conditions.

IV. Ascus and shorter paraphyses, mature; produced under drier conditions.

V. Ascus discharging spores; (*a*) normal, mature ascospores; (*b*) swelling for germination; (*c*) germination in water of freshly discharged ascospores, and of ascospore segments left dry for 7 days.

(III, IV, V to scale shown.)

of ochraceous-buff, -salmon, or -tawny shade, depending upon the age, substratum, and extent of diffusion of colour from the substratum or plectenchyma into the sporodochia. Normal cultures produce a carmine to Bordeaux coloration in starchy neutral media. Chlamydospores are absent; swollen segments present in the mycelium, and thick-walled segments in the plectenchyma should be distinguished.

Measurements of the perithecial stage of G. Saubinetii *from English cereals*, 1928–9.

On naturally infected wheat grains; brought to maturity under warm and moist conditions:

Perithecia, 200–250 × 190–210 μ.
Asci, average, 60 × 10·5 μ.
Ascospores, 3-septate, 20 × 3·5 μ, before signs of swelling; when slightly constricted at time of discharge, 20·8 × 4·5–5·0 μ.

On cooked wheat grains, brought to maturity on moist, sterile soil, temperature 6°–32° C., mainly 10°–25° C.; from single-conidium pure cultures.

Perithecia, 250–290 × 200–250 μ; average, 275 × 230 μ.
Asci, 60–82 × 10·4–13·0 μ; average, 78 × 11·1 μ; some retain a 1- or 2-celled pedicel, 5 μ or 10 μ long; asci shorter if less mature ones discharged under pressure.
Ascospores, 3-septate; smaller 1-septate occasionally present; 23·5–28·5 × 4·2–5·0 μ; average, 26·0 × 4·5 μ.

On living wheat and barley grains; inoculated from single-conidium pure cultures; grown under warm, moist conditions:

Perithecia, 250–260 × 220–240 μ.
Asci, 60–70 × 10·0–11·5 μ.
Ascospores, 3-septate, 20–30 × 4·2–4·8 μ; average, 25·0 × 4·25 μ.

The perithecia referred to above were crowded on a thin, mycelial plectenchyma (stroma); in artificial cultures they may be similarly crowded, or scattered singly on a loosely woven or open mycelial layer on the surface of the substratum. The perithecia are ovoid to subconical, black to the unaided eye and dark blue by transmitted light. The dark, thick-walled cells of the general peridium are replaced below by thinner-walled and smaller cells which merge gradually into the plectenchymatic mycelium. At the apex there is a more or less pronounced beak, the cells of which, in the immature perithecium, are almost colourless (Fig. 2, I). The cells of the outermost layer of the peridium project irregularly, as a rule, giving a verrucose type of perithecium; these cells

are more abundant and regular around the base of the beak, there forming a "collar." Wollenweber (5) states that on some substrata the stroma assumes the form of a Sphaerostilbe-like projection with the perithecia at the apex; that the perithecium is verrucose under very moist conditions; and that there is much variation in the general appearance and size of perithecia in pure cultures and much more so in field material (Fig. 2, II).

Asci are numerous (up to 100) in the perithecium, 8-spored, $1\frac{1}{2}$–2 seriate, with a few 3- or 4-celled paraphyses interspersed. The ascospores are fusiform, straight or, more commonly, dorsiventral, 3-septate; 1- or 2-septate spores occur occasionally in the same asci as the 3-septate, and the latter show some variation in size, the lowest spore in the ascus being usually the largest. The average size of 3-septate spores is $20–26 \times 3{\cdot}5–4{\cdot}5\mu$, with extremes of $20–40 \times 3{\cdot}4–5{\cdot}5\mu$.

Conclusions.

The characters detailed above show that the fungus in question is *G. Saubinetii* (Mont.) Sacc. The diagnosis agrees with Wollenweber's standard, except that in the present organism the ascospores are constantly slightly wider; whether this be due to "strain" or to technique will be evident when the organism has been compared with Continental and American forms under the same conditions. The variation is, however, not such as to throw any doubt on the identity of the fungus. It must now be recognised that *G. Saubinetii* occurs in English wheat and barley crops, but to what extent is at present unknown.

Summary.

1. The occurrence of *Gibberella Saubinetii* (Mont.) Sacc. on wheat and barley grown in England is recorded, but the extent to which it occurs in this country is not known.

2. The methods by which it was isolated and grown in pure culture are described.

3. The complete life cycle of this "strain" has been carried through from single-conidium and single-ascospore pure cultures.

4. The diagnostic characters are given, and the identification verified.

The writer tenders thanks to Dr G. H. Pethybridge for assistance rendered.

(Since writing the foregoing article the writer has isolated *G. Saubinettii* from the bases of wheat, barley and *T. monococcum* plants grown in the Eastern Counties. 23/12/29.)

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BACTERIAL "BLIGHT" OF WALNUTS IN BRITAIN

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(With Plate IV.)

Introduction.

During recent years an attempt has been made to encourage the cultivation of walnuts in this country (10, 11). With this end in view nursery trees of various varieties of walnuts have been collected at the East Malling Research Station for studying methods of propagation and for the selection of varieties suitable for cultivation in Britain (14). Several young trees (among others obtained for this purpose) were received from France and were planted up on the Station in March 1925. Early in September of the same year some of the leaves on one of these trees showed numerous small blackish spots. These spots on microscopic examination were seen to contain numerous bacteria-like rods and, as no other organism could be found associated with the spots, bacterial infection was suspected. One of the infected spots was therefore teased out in sterile water and isolation plates prepared. The first bacterial colonies to show up were white ones, but later yellow ones also appeared. The two organisms were isolated in culture and named *I a* and *I b*, the former from a yellow colony, the latter from a white one. In October lesions were found on the rachis of a leaf on the same tree. From one of these lesions plates were prepared and again white colonies appeared first, followed by yellow ones. Isolations were made from these and numbered *II a* (yellow) and *II b* (white).

In April 1926, using these four strains, inoculations were made, by puncturing, on leaves of a young walnut tree growing in a pot in the greenhouse. As these experiments will be described below it will be sufficient to state here that the punctures made with *I b* and *II b* (the white organisms) resembled control punctures, while those made with *I a* and *II a* developed a black discoloration of the tissues bordering on the punctures. The white organisms were therefore discarded and the yellow ones retained, as strains[1] *I* and *II*, for further study.

[1] The word "strain" is here used in a general sense to denote a series of cultures from one isolated colony.

The character of the blackened spots on the naturally infected leaves, and the presence in such spots of a yellow pathogenic bacterium, suggested that the disease was "bacterial blight" of walnuts, a disease not previously recorded in this country. Further study confirmed this, the evidence being presented below.

Distribution.

During the last decade of the nineteenth century Pierce investigated a bacterial disease which was causing considerable loss in the walnut groves of California, and in 1901 he published a brief description of "a new micro-organism pathogenic to *Juglans regia* and related species" (6). He named the organism *Pseudomonas juglandis* and proved its parasitism by obtaining positive results in inoculation experiments with pure cultures of his organism. During the next ten years the disease continued to be very serious in California and it was reported from the neighbouring State of Oregon and also from Texas. Pierce's work was followed by that of R. E. Smith and his collaborators, and in 1912 a detailed account of the disease and of the causal organism was published (9). By 1917 it had been observed by Waite (13) and by McMurran (3) in certain eastern states, viz. Louisiana, the District of Columbia, Maryland, Delaware, Pennsylvania, and New York.

The disease has also been recorded from New Zealand (1,2) and Australia (5), and in 1922 Pole-Evans (7) mentioned "walnut blight" among other diseases as "very prevalent" in South Africa. In Europe it has been reported from Switzerland (4), Italy (8), and Holland (12)[1].

These "records," however, are mostly short notices of the occurrence of the disease, the diagnoses being, presumably, based on the symptoms shown by the diseased organs, and there appears to be no detailed account of isolation and inoculation work apart from that carried out by the Californian investigators, and by McMurran, who made isolations, carried out inoculation tests, and stated, without giving details, that "the cultural studies so far made from these isolations coincide with those made by Smith and by Pierce."

The writer has not yet seen any report of the occurrence of this disease in France. The appearance of the disease at East Malling during the first season after the tree was planted up suggested that the tree was infected when received from France, and a note to that effect was published in 1927 (15). In August of that year the writer received a letter from Mons. J. Dufrénoy, of the Station de Pathologie végétale, Paris,

[1] While this article was in the press the author found a reference to the occurrence of the disease in Chile (see *Biological Abstracts*, Vol. III. p. 49).

who desired to know from which nursery the tree was received. On being informed, Mons. Dufrénoy wrote to say that he had visited the nursery in question as recently as the spring of 1927 without noticing any trouble on the walnut trees or seedlings.

It must be left an open question, at present, therefore, whether there is any danger of introducing "blight" infected trees from France. It may be of interest to note in passing, however, that Smith found what appeared to be typical bacterial blight lesions on walnut scions imported into California from France in 1907, and although he was unable to isolate the organism from such lesions, it was observed that the scions developed typical blight on the young growth under conditions suggesting that infection had come from the imported scion wood.

Observations on the disease at East Malling.

The spots on the leaf-blades were mostly 0·5 mm. or less in diameter and generally angular in shape, being bordered by the smaller veins. Where the spots were numerous they became confluent and sometimes caused large brown patches of withered tissues. In some cases the spots were most crowded in those regions of the leaf bordering on the midrib and larger veins, and at the tips of drooping leaflets. This suggested that the organism was disseminated mostly by rain, as rain water falling on the leaves would tend to flow towards, and accumulate at, such places during showers. The conspicuous infection of the leaf-tips was noticed particularly on a few trees grown in the greenhouse; these trees were sprayed daily with water during the warmer months. On the petioles the infection spots were elongated blackened lesions up to 2 cm. long.

Early in June 1926 the stem of a young tree about one foot high, growing in a pot in the greenhouse, bore three elongated blackened lesions. This stem consisted of that year's growth only and corresponded therefore to a shoot of the current year. Two of the lesions were near the base while the other was starting at a node near the middle of the stem; about a fortnight later the latter had completely encircled the stem. Culture plates prepared from this uppermost canker yielded yellow colonies only, similar to those obtained from the leaf-spots. From one of these colonies strain *III* was isolated.

In 1928 several trees about 6 feet high, growing in the greenhouse, and bearing a few fruits, were found to be infected; the typical spots were seen on a number of leaves and on one tree two nuts showed black lesions near the style end. The nuts, when infection was noticed, were about 1 cm. long and the lesions were 2 to 3 mm. in diameter. From these blackened spots isolation plates were prepared. One of the spots

yielded yellow colonies only, the other gave yellow colonies together with white ones. Strains were isolated from the yellow colonies and numbered *IV* (1) from one nut, *IV* (2) from the other.

The tree on which the disease was first observed was one of a batch received at East Malling in January 1925; the trees on their arrival were "heeled in" the ground and planted out in their permanent quarters in March. As already mentioned, the leaf-spotting was seen on this tree during the late summer of the same year. The variety was Chaberte. The disease appeared again on this variety in the following year. In 1927 (a rather wet season) the disease was more pronounced on Chaberte, some of the leaves bearing very numerous coalescing spots so that the edges of the leaves were brown and curled upwards. Similar spotting was found on the leaves of varieties Treyve, Meylannaise, Vourey, Franquette and Gladys, the last named being severely infected. In 1928 (a summer drier than in 1927) it was observed on Mayette (very little infection), Parisienne, Chaberte (again considerable spotting), Meylannaise, and Gladys (very little). These are all varieties of *Juglans regia*.

Inoculation experiments.

The inoculations were carried out on leaves and shoots of a young tree growing in a cool greenhouse.

Exp. 1. In this preliminary experiment inoculations were made on leaves by puncturing and placing drops of a suspension of the organism on the punctures, April 15th, 1926. The strains used were:

I a. Yellow organism from a leaf spot.
I b. White " " "
II a. Yellow organism from a lesion on petiole.
II b. White " " " "

For each strain one leaf was selected and the punctures made on the five terminal leaflets, *i.e.* the apical leaflet and the next two pairs; four punctures were made on each leaflet. The punctures on one leaflet of each pair were inoculated, those on the other leaflet being left as control. On the terminal leaflet two punctures on one side of the midrib were inoculated, the two on the other side left as control. Evidence of infection was not apparent until about three weeks afterwards. By May 7th some of the spots inoculated with *I a* and *II a* were more conspicuous than the rest by reason of a border of blackened cells. On June 7th the spots inoculated with *I b* and *II b* showed no blackening whatever. Of those inoculated with *I a* all except one showed a blackened border together with, in most

cases, a yellow zone outside this. The spots inoculated with *II a* showed similar infection; in this case the two control punctures on the terminal leaflet showed a little blackening, but the rest of the control spots showed no trace of infection. Thus, of 20 spots inoculated with the yellow organisms 19 became infected, 20 inoculated with the white organisms were not infected, and of 40 control spots all except two showed no sign of infection, and these two being in close proximity to two infection spots were probably secondarily infected from the latter.

Exp. 2. Strains *I* and *II* were inoculated into leaves and young shoots at punctures, June 6th, 1926. With each strain three shoots were inoculated at two punctures each, one puncture a few millimetres below the growing point and the other about one centimetre lower down. Three other shoots were punctured but not inoculated, as controls. Strain *I* only was used on the leaves. Two leaves were inoculated, one (*a*) not quite unfolded, the other (*b*) was the next older leaf on the same shoot. On leaf (*a*) four punctures were inoculated on each of four leaflets on one side of the rachis; on leaf (*b*) two leaflets were inoculated at 20 punctures each. The corresponding leaflets on the opposite side of the rachis were similarly punctured but not inoculated.

By July 15th the shoots showed infection at all the inoculated spots, as seen by a discoloration round the punctures, the degree of infection varying with the thickness of the shoots, *e.g.*

Shoots about	2 mm.	in diameter:	lesions 2 × 1·5 mm.
,,	4 mm.	,,	,, 4 × 3 mm.
,,	5 mm.	,,	,, 6 × 4 and 8 × 4 mm.

On the younger leaf (*a*) every inoculated spot was bordered by a black rim round which was a yellow zone. On leaf (*b*) 18 of the 20 inoculated spots showed similar evidence of infection. The control punctures showed no blackening.

On July 15th one of the shoots inoculated with strain *I* was cut off and from the two lesions isolation plates yielded yellow colonies from which strains *I R* 1 (from one lesion) and *I R* 2 (from the other) were isolated.

Exp. 3 was carried out to test the parasitism of strains *I R* 1, *I R* 2 and *III*. The inoculations were made, as before, by puncturing leaves and inoculating at the punctures; the inoculated punctures numbered 20, 28 and 20 respectively for the three strains, while 60 control punctures were made, some of them on leaflets on the same rachis as inoculated leaflets.

All the inoculated punctures developed the symptoms shown in the earlier experiments, while the control punctures showed no discoloration.

Exp. 4. The strains used in Exp. 3 were inoculated into shoots by puncturing, the number of inoculations being 6, 2, and 2 respectively, with 12 control punctures. All the inoculated punctures gave rise to discoloured lesions while the control punctures remained as brownish dots with no further discoloration.

Exp. 5 was carried out to test the parasitism of strains *IV* (1) and *IV* (2). Each strain was inoculated at four punctures into shoots. All the inoculated punctures became infected, while control shoots showed no infection.

Description of the organism causing walnut blight.

The morphology of the organism and its physiological reactions on various culture media were studied for comparison with those described by Pierce (6) and by Smith (9) for *Pseudomonas juglandis* Pierce, particularly with regard to the "salient characters" which enabled Smith to give the organism the group number 211.3332513. A culture of *Ps. juglandis* was received from California[1] and tests were applied, simultaneously, to those strains isolated at East Malling which had been proved to be pathogenic, and to the Californian strain.

Morphology.

The organism, taken from nutrient agar cultures 24 hours old, fixed with formalin, stained with methyl violet and mounted in euparal was seen to consist of rods 1·2 to 2·2μ long in those showing no division, while those in process of dividing were 2·0 to 3·3μ long; their width was 0·3 to 0·5μ. These dimensions are a close approximation to those given by Smith who measured rods taken directly from leaf-spots and found them to measure 1·5 to 3·01 $\times$ 0·3 to 0·51μ.

The rods were readily stained by carbol fuchsin, methyl violet, gentian violet, and aniline gentian violet, but rather faintly by methylene blue and by Gram's stain.

In hanging drops of nutrient broth, from cultures 24 hours old, many of the rods, single and double, were seen in active motion. Taken from young nutrient agar cultures and stained by Caesares-Gil's method the organism showed a single wavy polar flagellum three to four times the length of the rod.

[1] The writer is indebted to Mr C. O. Smith for sending this culture of *Pseudomonas juglandis* from California.

Taken from a five-week-old culture on potato and stained with Ribbert's capsule stain, the rods showed the "hyaline membrane" mentioned by Smith.

No spores have been observed and to obtain confirmation as to their absence a heat test was applied. Bacterial slime was taken from fifteen-day-old cultures on nutrient agar and shaken up in tubes of sterile water, giving a slightly turbid suspension. In each case a loopful of this suspension was transferred to a tube of nutrient broth as a control to show viability. The tube containing the suspension in water was then heated for 10 minutes at 80° C. and after cooling another tube of nutrient broth was inoculated from it. This test was applied to all the eight strains and in every case the tube inoculated before heating the organism produced a normal culture, while that inoculated after the heating remained sterile.

CULTURAL AND PHYSIOLOGICAL CHARACTERS.

The walnut organism grows well in most of the media used. Poured plates of nutrient agar produce three forms of colonies according to their situation in the medium. The surface colonies are yellow and generally circular, raised, smooth and shining. Those embedded in the medium are lenticular. Smith in describing these "deep colonies" writes, "They vary somewhat in shape, being biconvex, angular or oval to circular in outline." This description is misleading, for if the colonies are examined with a lens near the edge of the plate where the same colonies can be viewed from above and from the side, it will be seen that the embedded colonies are each circular *and* biconvex, their apparent shape being due to their orientation in the agar, since they are tilted at different angles to the surface of the plate. Colonies growing at the bottom of the medium in plate cultures are circular in outline and very faintly coloured.

The surface colonies of nutrient agar plates show some variation in shape. Whether this variation is a result, in some cases, of "saltation" is a point which has not yet been examined closely for this organism, but it may be mentioned that in one strain (*I R* 1) two types of surface colonies appeared in poured plates after the strain had been in culture for some two and a half years; one type was raised, smooth, and circular, the other larger, less raised, but umbonate, and rather irregular in shape. The former appears to be the more general type met with and is probably that seen by Smith. That the umbonate type is a variant of the other, and not a contamination, appears probable from the fact that the culture reactions of the mixed strain showed no divergence from the rest of the

strains, and in tests where the two types have been studied after isolation they have given similar results, except in the form of the colonies.

On nutrient agar slopes there develops a smooth glistening yellow growth only slightly raised, but in nutrient agar containing 5 per cent. saccharose growth is more vigorous and the bacterial mass is distinctly raised.

Stabs in nutrient agar produce slightly raised yellow plates at the surface of the medium and the path of the stab itself becomes "tuberculate echinulate" as noted by Smith.

In nutrient gelatine cultures the medium is slowly liquefied. In stab cultures kept at 20° C. liquefaction may be seen beginning within two days; about the fourth day liquefaction is crateriform, but later (as no liquefaction occurs in the stab itself) it becomes stratiform and after a month extends downwards from the surface for about 2 cm. The liquefied gelatine remains almost clear but a copious yellow sediment collects below. In one test liquefaction was not complete until about three months after inoculation. The absence of liquefaction along the stab, together with the retarded development in the later stages of these gelatine cultures, denotes that the organism is strongly aerobic.

In liquid media growth is most vigorous at the surface, usually with the formation of a ring or pellicle. In nutrient broth C. O. Smith noted a ring but no pellicle. In the present series of tests a thin fragile pellicle was sometimes produced in broth cultures, and a pellicle generally appeared on the addition of a carbohydrate to the broth. In nutrient broth with 5 per cent. saccharose growth was very vigorous and a thick pellicle was produced which in old cultures reached a thickness of 1 cm. or more. In one batch of cultures in this medium, when eight months old growing at room temperature, the contents of each tube were a mass of gelatinous bacterial growth, so that the tubes could be held inverted without the contents flowing out. Inoculations into nutrient broth from these cultures when nine months old gave rise to typical growth, showing that such cultures retain their viability for at least nine months.

Neither acid nor gas was produced in litmus broth cultures containing dextrose, lactose, saccharose or glycerine, the test being carried out in Durham's tubes. In these cultures no growth could be detected in the inner (inverted) tube.

In fermentation tubes containing 1 per cent. saccharose in nutrient broth (without indicator) there was copious growth in the open arm. On casual examination there was apparently no clouding of the closed arm, but close examination after seven or eight days showed a slight

opalescence of the liquid in the closed arm. This opalescence was never very noticeable and even after a month there was no distinct cloudiness in the closed arm.

Tests for nitrate reduction gave negative results. The cultures for this test were in broth containing 0·1 per cent. potassium nitrate and the tests for nitrite were applied at the end of seven days in one series and twelve days in another. The contents of each culture tube were divided into two parts; to one part the iodine-starch test was applied, the method used by Smith; the more sensitive naphthylamine test was applied to the other part. In no case was there any evidence of nitrite production, thus confirming Smith's results.

Tests for the formation of indol were made in cultures in peptone water (1 per cent. Bacto-peptone) and in "tryptophane broth." At the end of fourteen days the contents of each tube were divided into two parts; to one half was applied the nitrosoindol test (as used by Smith) and to the other half the Ehrlich-Böhme test. Smith (using Witte's peptone) obtained "a strong indol reaction" on applying the nitrosoindol test and then heating. In the tests carried out by the present writer the results were all negative; no pink colour developed even after heating, though browning occurred in some tubes to which the nitrosoindol test had been applied.

There was good growth on sterilised potato plugs in test tubes; the bacterial streak, when six days old (growing at room temperature) was bright yellow (mustard yellow to primuline yellow), while the potato itself was turning grey.

On sterilised potato slices 4 to 5 mm. thick, incubated at 25° to 26° C., there was also good growth accompanied by a bluish-grey discoloration of the potato. Within a few days a narrow whitish zone appeared round the bacterial growth. This zone gradually became more conspicuous and its width was 5 to 7 mm., in some cases, when the cultures were a month old. It appears to be the "fermentation zone" noticed by Pierce and by Smith, though neither of these workers records the fact that the potato under the bacterial slime either sinks or is digested away (probably the latter) so that the yellow mass lies in a crater-like hollow which in the experiments here recorded was about 2 mm. deep, in cultures four weeks old, thus extending about halfway through the slice. The "white" zone was seen to be the side of the depression showing between the bacterial mass and the edge of the crater. The whiteness appears to be chiefly due to the reflection of light from the sides of the crater as its intensity (particularly in the early stages) varied with the change in the incidence

of light, as was seen when the plate was turned round horizontally in front of a window. Particles taken from inoculated slices after three weeks, and tested with iodine gave a less pronounced starch reaction (particularly if taken from the bottom or sides of the "crater") than particles from control slices. This indicates hydrolysis of starch as noted by Pierce and by Smith.

The action of the organism on starch was more clearly shown, however, by employing other methods[1]. Streaks were made on the surface of plates of nutrient agar containing 0·2 per cent. soluble starch. After six days the plates were flooded with iodine solution; no blue coloration appeared showing that all the starch had been hydrolysed. In this test four streaks were made on each plate, the streaks being about 1·5 cm. apart. Other plates were then prepared for examination of the rate of progress of the action. Only one streak was made on each plate and the iodine test applied at the end of two days and five days respectively. After two days the clear unstained zone round the streak extended 4 to 6 mm. from the edge of the streak; after five days the zone was 17 to 20 mm. in width.

Further tests were made in tubes of nutrient broth containing soluble starch (0·2 per cent.) and others of nutrient broth with rice starch (0·03 per cent.). On adding iodine to the tubes at the end of seven days the inoculated tubes gave no starch reaction at all, while the contents of control tubes were stained a deep blue.

In milk, litmus milk, and methylene blue milk the results were very similar to those obtained by Smith. No acid was produced; a soft curd was formed which was slowly digested, so that the milk (if previously uncoloured) became a clear translucent yellowish liquid with a distinct pellicle at the surface and a yellowish sediment at the bottom. In some litmus milk cultures the indicator was decolorised, in others the "wine-coloured appearance," mentioned by Smith, eventually appeared. The methylene blue was reduced so that in a few days the tubes resembled the plain milk tubes; the blue colour reappeared on heating the tubes.

Milk containing bromo-cresol purple as indicator was also used; the production and solution of the casein curd occurred as in plain milk, but the final liquid was purple in colour, denoting that its reaction was alkaline.

In Cohn's solution (formula as given by E. F. Smith[2]) C. O. Smith records "after three months there was no growth." In tests carried out

[1] See *Manual of Methods for Pure Culture Study of Bacteria* issued by the Society of American Bacteriologists, p. C 27.

[2] *Bacteria in Relation to Plant Diseases*, I, 197.

by the present writer some growth could be detected in every case in from eight to fifteen days. The turbidity never became very evident and might be overlooked except by a careful comparison of inoculated tubes with control tubes. The tubes of the first series were inoculated from nutrient broth cultures and it was thought that possibly sufficient organic food material was carried over in the loop to enable the organism to make feeble growth. To test this point further another batch of tubes of this medium was prepared and inoculated. In order to avoid carrying over traces of broth during the operation, one set of tubes was inoculated from the cultures of the former test in Cohn's solution, and another set was inoculated from a suspension of the organism obtained by taking a little of the growth from a nutrient agar slope and shaking it up in a tube of sterile distilled water. All eight strains (including that received from C. O. Smith) were tested. In every case a slight turbidity could be detected in eight days. Six days later this turbidity, though still slight, was unmistakable when the tubes were compared with control tubes of the same batch, and in the inoculated tubes there was a little sediment which on shaking floated up as stringy flocculi. Inoculations into nutrient broth, from cultures in Cohn's solution one month old, gave rise to typical growth so that there is evidence that the walnut blight organism is not only able to grow (though feebly) in Cohn's solution but that it retains its viability for at least a month in this medium, which contains inorganic nitrogen and organic acid but no carbohydrate, and has a relatively high acidity (pH about 5·2).

The morphological and cultural characteristics of the organism causing bacterial blight of the walnut trees at East Malling correspond very closely, therefore, with those of *Pseudomonas juglandis* Pierce[1], its group number being the same as that given by C. O. Smith for *Ps. juglandis*, viz. 211.3332513. According to the "Brief Characterization" of "Primary Characters" of the more recent "Descriptive Chart" of the Society of American Bacteriologists its "Index Number" is 5021–31105–1000.

The discrepancies from Smith's description are probably due to differences in technique since no constant difference has been observed between the various strains isolated at East Malling and the strain supplied by C. O. Smith from California.

[1] According to the recent classification of bacteria adopted by the Society of American Bacteriologists its name would be *Phytomonas juglandis*, but it is not included in either the 1st (1923) or 2nd edition of Bergey's *Manual of Determinative Bacteriology*. Erwin F. Smith places it in the genus *Bacterium* (Cohn emend.). See his *Bacteria in Relation to Plant Diseases*, I, 171.

SUMMARY.

1. An organism has been isolated from lesions on leaves, shoots, and fruit of young walnut trees at the East Malling Research Station, Kent.

2. This organism was proved to be pathogenic by positive results obtained from inoculation on walnut leaves and shoots.

3. It corresponds closely to descriptions of *Pseudomonas juglandis* and is therefore referred to that species.

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EXPLANATION OF PLATE IV

Fig. 1. A walnut leaflet with bacterial spotting.

Fig. 2. Leaf spots as seen by means of a hand lens ($\times 5\frac{1}{2}$).

Fig. 3. Stem of a young walnut tree showing two bacterial lesions (natural infection).

Fig. 4. Walnut leaflets artificially punctured; on the left punctures inoculated with a pure culture of *Pseudomonas juglandis*, on the right punctures not inoculated. Note the black rim round the inoculated punctures, outside which there is usually a zone of pale-coloured leaf tissue.

Fig. 5. Four bacterial lesions on a walnut branch, the result of inoculation at punctures.

(*Received May* 15*th*, 1929.)

Fig. 1.

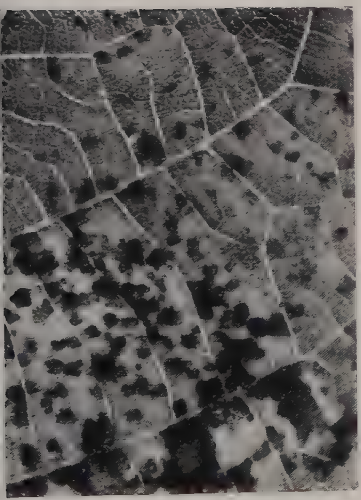

Fig. 2.

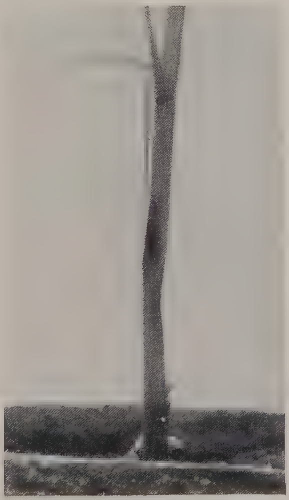

Fig. 3.

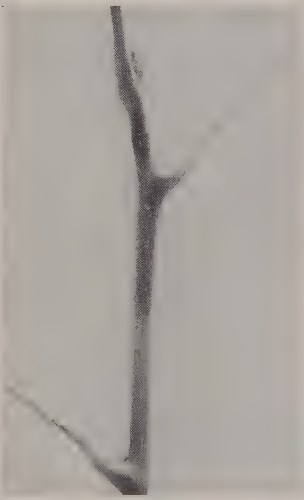

Fig. 5.

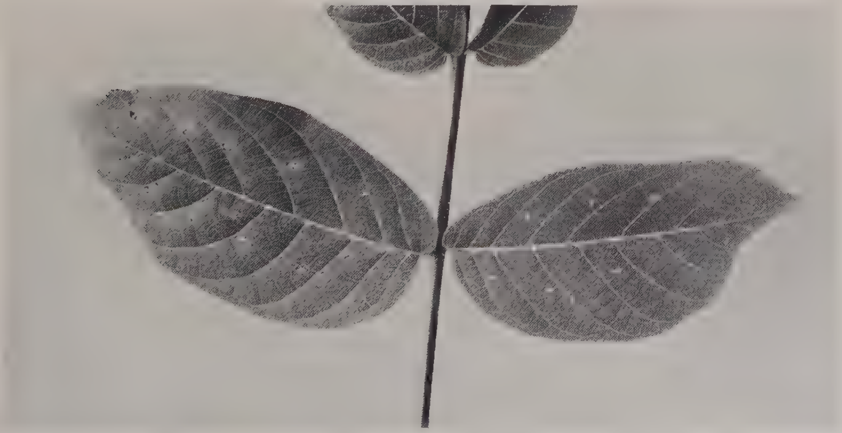

Fig. 4.

WORMALD.—BACTERIAL "BLIGHT" OF WALNUTS IN BRITAIN (pp. 59–70).

THE RELATION OF ATMOSPHERIC TEMPERATURE AND HUMIDITY TO TOMATO LEAF MOULD (*CLADOSPORIUM FULVUM*)

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Leaf mould (*Cladosporium fulvum* Cke.) is a serious disease of glasshouse tomatoes. Resistant varieties offer the best means of control, but until these are available growers must look to a reduction in the atmospheric temperature and humidity of the glasshouses to lessen its severity. The purpose of this investigation was to obtain experimental data concerning the relation of atmospheric temperature and humidity to leaf mould, which would assist growers to control the disease by cultural methods.

Experimental methods.

In these experiments healthy tomato plants about nine inches high were inoculated on the lower surface of the leaves with a suspension of spores in water. Spores were obtained from diseased leaves and their viability tested by suitable methods. The various series of each experiment included not less than seven plants. The experiments were made in small glass chambers, and the average temperature and relative percentage humidity were calculated on a two-hour basis from continuous records taken by thermo-hygrographs which were standardised frequently. In temperature experiments, the chambers were maintained at approximately 95 per cent. humidity and were placed where temperature variations were small. In humidity experiments, made whenever possible under optimum temperature conditions, control was obtained by sulphuric acid and, after inoculation, the plants were allowed to dry before being placed in the chambers.

Plants were examined on alternate days and the development of leaf mould was denoted as follows:

Degree of sporulation

- *A* 1. Faint grey mould. Little or no sporulation.
- *A* 2. Marked grey mould. Definite sporulation.
- *A* 3. Intense sporulation denoted by appearance of brown mould.
- *A* 4. Very intense sporulation—large patches of brown mould.

Area covered by the disease

- *B* 1. Numerous scattered spots of leaf mould.
- *B* 2. Leaf mould covering approximately half of each leaflet.
- *B* 3. Leaf mould covering more than half of each leaflet.

In the tables given later "no disease" implies that none was visible to the naked eye while "disease appearing" denotes a milder attack than the *A* 1, *B* 1 standard. Intermediate stages are denoted by *A* 1–2 or *B* 1–2, etc. It is impracticable to publish all the records taken, but the data given in the tables indicate the development of the disease under the experimental conditions.

Temperature relations.

The relation of temperature to leaf mould.

Inoculated plants were grown in chambers maintained at average temperatures of 10° C., 13° C., 17° C., 20° C. and 23° C. respectively. The results, given in Table I, show that leaf mould developed most severely at 23° C., and that at 17° C. and 20° C. the plants were severely diseased, while those at 10° C. and 13° C. remained apparently healthy. Microscopic examination showed that the latter plants were infected. Thus, of the temperatures tested, 23° C. was nearest to the optimum for leaf mould, while at 13° C. it developed very slowly even under humid conditions.

Table I.

The relation of temperature to leaf mould.

Date of inoculation	No. of plants	Av. temp. ° C. 11–18. v. 28	18–26. v. 28	11–26. v. 28	Result 19. v. 28	23. v. 28	26. v. 28
11. v. 28	15	23	22	23	Disease appearing	15 plants, *A* 3, *B* 2	3 plants, *A* 3, *B* 2 12 ,, *A* 4, *B* 3
,,	15	20	20	20	No disease	13 ,, *A* 2, *B* 1 2 ,, *A* 3, *B* 2	4 ,, *A* 2, *B* 1 11 ,, *A* 3, *B* 2
,,	15	18	17	17	,,	13 ,, *A* 2, *B* 1 2 ,, *A* 3, *B* 2	14 ,, *A* 3, *B* 2 1 ,, *A* 4, *B* 3
,,	15	14	13	13	,,	No disease	No disease
,,	15	11	10	10	,,	,,	,,

The relation of temperature to infection.

This was determined by placing inoculated plants in chambers, maintained at average temperatures of 14° C., 17° C. and 22° C. respectively, for four days only, and afterwards moving them into a glasshouse. On moving the plants, controls were inoculated and placed beside them. Since the controls remained almost healthy it was concluded that scarcely any infection occurred on the treated plants after they were moved into the glasshouse. The results, given in Table II, show that severe infection occurred in four days at 14° C., 17° C. and 22° C., which indicates that

the amount of infection is practically equal over a wide range of temperature if sufficient time is allowed. Leaf mould, however, appeared first on the plants at 22° C., which suggests that this temperature, which is within the optimum for spore germination, is the most favourable for infection.

Table II.

The relation of temperature to infection.

Date of inoculation	No. of plants	Av. temp. ° C. during exposure period (15–19. vi. 28)	Result 23. vi. 28	27. vi. 28	3. vii. 28
15. vi. 28	12	22	Disease appearing	5 plants, *A* 2, *B* 1 7 ,, *A* 2, *B* 2	12 plants, *A* 4, *B* 3
,,	9	17	No disease	9 ,, *A* 2, *B* 1	9 ,, ,,
,,	12	14	,,	8 ,, *A* 2, *B* 1 4 ,, *A* 2, *B* 2	12 ,, ,,

It is worth noting that all the treated plants in the above experiment developed a severe attack of leaf mould after they were moved into the glasshouse, while the inoculated control plants, also in the glasshouse, remained almost healthy for several days after the experiment was concluded. This result suggests that after infection has occurred, a severe attack may develop under conditions which almost inhibit infection. This was tested by inoculating three plants, each three feet high, and placing them in a moist chamber for three days to ensure infection. They were afterwards moved to a sunny position outside, at which time three control plants were inoculated and placed beside them. Three weeks after inoculation the treated plants had developed large patches of leaf mould on numerous leaflets, while the controls, four weeks after inoculation, had only two faint spots of disease present.

The effect of temperature upon leaf mould subsequent to infection.

Inoculated plants were placed in a moist chamber to ensure infection and were afterwards moved into chambers at 15° C., 20° C. and 22° C. respectively. The results showed that leaf mould developed very rapidly at 20° C. and 22° C., while at 15° C. it progressed very slowly, being scarcely visible twelve days after inoculation. The results suggest an explanation of the epidemics so familiar to growers. During cool weather much infection probably occurs but the disease develops slowly, so that the crop appears healthy. If at this stage warmer conditions intervene, an apparently healthy crop is transformed into a severely diseased one within a few days.

The relation of temperature to sporulation.

In this experiment inoculated plants were grown in a glasshouse until leaf mould was just visible, at which stage the various series were placed in chambers at 18° C., 22° C. and 29° C. respectively. Sporulation was most abundant at 22° C. At 18° C. and 29° C. the disease progressed considerably, but these temperatures were respectively below and above the optimum for sporulation.

Humidity relations.

The relation of humidity to leaf mould.

In this experiment, made under optimum temperature conditions, inoculated plants were grown in chambers maintained at average humidities of 95, 80 and 65 per cent. respectively. Leaf mould was less severe at 80 than at 95 per cent. humidity, and was almost absent at 65 per cent. humidity fifteen days after inoculation. It was evident that the humidity must be below 80 per cent. to prevent leaf mould when optimum temperature conditions obtain. Examination of the plants at 65 per cent. humidity failed to reveal any germinating spores.

The relation of humidity to infection.

Inoculated plants were placed in chambers at 95, 80 and 71 per cent. humidity respectively for four days only, which allowed ample time for infection, and were afterwards moved into a glasshouse. Inoculated controls showed that scarcely any infection occurred on the treated plants after they were moved into the glasshouse. The results, given in Table III, show that severe infection occurred at 95 and 80 per cent. humidity and that it was slight at 71 per cent. humidity. Similar results were obtained

Table III.

The relation of humidity to infection.

Date of inoculation	No. of plants	Period of exposure (days)	Percentage humidity during exposure period: Abs. max.	Abs. min.	Av.	Temp. ° C. during exposure period: Abs. max.	Abs. min.	Av.	Result: 15. viii. 28	18. viii. 28	20. viii. 28
7. viii. 28	8	4	97	93	95	22	19	21	8 plants, *A* 1, *B* 1	4 plants, *A* 2, *B* 2 4 ,, *A* 3, *B* 2	8 plants, *A* 4, *B* 3
,,	8	4	86	79	80	22	19	21	No disease	2 ,, *A* 1, *B* 1 6 ,, *A* 2, *B* 1	4 , *A* 2, *B* 1- 4 ,, *A* 3, *B* 2-
,,	8	4	80	64	71	22	19	21	,,	3 ,, Disease appearing	6 ,, Disease appeari

in further experiments; it is concluded that infection is rare at 70 per cent. humidity under optimum temperature conditions (22° C.). At 18° C., however, a humidity of 78 per cent. retarded disease development and reduced the amount of infection considerably.

It is worth noting that the plants exposed to 95 per cent. humidity for four days subsequently developed a severe attack of leaf mould in the glasshouse, *i.e.* under conditions which, as the controls showed, almost inhibited infection. Since the temperature of the glasshouse was favourable to the disease, the result indicates that a lower humidity is required to check leaf mould development than to prevent infection.

The effect of humidity upon leaf mould subsequent to infection.

In this experiment inoculated plants were placed in a moist chamber to ensure infection and were afterwards moved into chambers maintained at average humidities of 95, 78 and 57 per cent. respectively. Leaf mould developed most severely at 95 per cent. humidity, while at 78 and 57 its progress was appreciably retarded.

The relation of humidity to sporulation.

This was determined by growing inoculated plants in a glasshouse until the disease was just visible and then placing them in chambers at 92, 78 and 58 per cent. humidity. The results showed that abundant sporulation occurred at 92 and 78 per cent. humidity, while at 58 per cent. the disease spots dried out and produced very few spores.

The effect of humid night conditions upon leaf mould.

Continuous records, taken among the plants in large glasshouses at intervals from May to August (1928), showed that the humidity exceeded 90 per cent. for several hours each night. The effect of such conditions upon leaf mould was tested by growing inoculated plants in a well-ventilated glasshouse during the day and moving them into chambers, maintained at different humidities, from 6.0 p.m. to 6.0 a.m. each night. The average night temperature was 19° C. The results showed that plants exposed to humidities exceeding 75 per cent. each night were severely attacked.

To determine the maximum period for which plants could be exposed to humid conditions and remain healthy, inoculated plants were placed in a moist chamber for 0 (controls), 4, 8 and 12 hours each night. When

not in the chamber the plants were placed in a dry position. The results, given in Table IV, show that all plants developed severe leaf mould except the controls.

Table IV.

The effect of humid night conditions upon leaf mould.

Date of inoculation	No. of plants	No. of hours in moist chamber each night	Temp. ° C. of moist chamber each night: Abs. max.	Abs. min.	Av.	Result: 15. viii. 28	18. viii. 28	23. viii. 28
7. viii. 28	12	12	21	15	19	12 plants, *A* 1, *B* 1	6 plants, *A* 3, *B* 3 6 ,, *A* 4, *B* 3	12 plants, *A* 4, *B* 3
,,	12	8	21	15	19	5 ,, Disease appearing	12 ,, *A* 2, *B* 1	12 ,, *A* 4, *B* 3
,,	12	4	21	15	19	7 ,, ,,	12 ,, *A* 2, *B* 1	4 ,, *A* 3, *B* 2-3 8 ,, *A* 4, *B* 3
,,	12	0 (controls)	21	15	19	No disease	No disease	Few disease spots appearing

It is concluded that humid night conditions are largely responsible for the prevalence and severity of tomato leaf mould in commercial glasshouses, and that their harmful effect is not counteracted by dry day conditions. Some growers attempt to reduce the humidity by keeping the temperature high at night, but unless the humidity is sufficiently reduced by this practice it is clear that the higher temperature will favour leaf mould.

The relation of watering to the disease.

Overhead watering. The high humidity and wetting of the foliage caused by this practice are considered favourable to leaf mould by many growers. Records show, however, that the former does not persist long enough to affect the disease if watering is done early on fine days and ample ventilation is given. To determine if wetting of the leaves affected leaf mould, plants were inoculated and allowed to dry, after which they, excluding controls, were gently atomised with water each morning and, in some cases, each evening. Morning treatment caused the foliage to remain wet for about three hours. The experiments were made on small pot plants in a glasshouse and were repeated at intervals over a period of two years under various climatic conditions. The results showed that the treatment had no appreciable effect upon the disease. It may be contended that results with small plants are not applicable to dense crops in glasshouses. On the other hand, overhead watering is not practised daily nor is the foliage so completely wetted as in the above experiments.

Soil watering. The development of leaf mould on turgid plants was compared with that on plants beginning to droop. The experiments were repeated at intervals over a period of two years and are divided into two groups. In the first, inoculated turgid and drooping plants were placed in a moist chamber to ensure infection and afterwards moved into a glasshouse. The drooping plants did not recover in the moist chamber. In the second group, plants were placed in the glasshouse immediately after inoculation. Typical results are given in Table V.

Table V.

The development of leaf mould on turgid and drooping plants.

Treatment	Total no. of plants	Result: Turgid	Result: Drooping
Placed in moist chamber for 3 days after inoculation and afterwards grown in glasshouse	30	15 plants, *A* 4, *B* 3	9 plants, *A* 2, *B* 1 6 „ *A* 3, *B* 2–3
Ditto	12	6 „ *A* 4, *B* 3	6 „ A 2–3, *B* 1
Placed in glasshouse immediately after inoculation	30	15 „ *A* 4, *B* 3	5 „ No disease 10 „ A 1–2, B 1
Ditto	12	5 „ *A* 2, *B* 1 1 „ *A* 2, *B* 2	6 „ No disease
Ditto	12	6 „ *A* 4, *B* 3	6 „ „

In all experiments turgid plants were more susceptible to leaf mould. In the second group, *i.e.* where the plants were placed in the glasshouse immediately after inoculation, infection was very severe on turgid plants but was slight on drooping plants. This result suggests that the higher humidity around the turgid leaves caused spore germination and that its decrease by means of air currents would lessen the severity of leaf mould.

The development of leaf mould at various seasons of the year (1928).

These experiments were made in a glasshouse. Each month twenty plants were inoculated; ten were placed in a moist chamber to ensure infection and afterwards moved into the glasshouse, while ten were placed in the glasshouse immediately after inoculation. Both series yielded similar results and only those from the latter series will be considered. The average temperature and relative percentage humidity were calculated on a four-hour basis from continuous records. The results, given in Table VI, show that seasonal variations in the severity of leaf mould are related to temperature. The disease was severe when the average temperature exceeded 18° C., *i.e.* from May to August, and was slight at

temperatures below 15° C., *i.e.* from November to March, despite the favourable humidity conditions.

Table VI.

The development of leaf mould at various seasons of the year (1928).

Month	No. of plants	Av. temp. °C.	Average percentage humidity	Result: After 2 weeks	Result: After 3 weeks
January	10	12	88	No disease	No disease
February	10	13	85	,,	,,
March	10	14	84	,,	Little disease
April	10	15	77	Little disease	,,
May	10	19	81	10 plants diseased	10 plants severely diseased
June	10	20	78	,, ,,	,, ,,
July	10	22	75	,, ,,	,, ,,
August	10	18	79	,, ,,	,, ,,
October	10	15	85	Little disease	,, ,,
November	10	12	85	No disease	Little disease

Control methods.

In England tomatoes are planted in February or March. Towards the end of April a dense mass of tender foliage has developed, heavy watering is practised frequently, higher temperatures begin to operate and periods of high humidity occur owing to inadequate ventilation of the glasshouses. Leaf mould appears in May and often spreads rapidly throughout the crop. Sometimes the plants are killed but usually they survive owing largely to severe pruning of the diseased foliage.

Suggestions for controlling leaf mould by cultural methods are given below.

1. *Temperature.* The control of leaf mould by means of temperatures above 22° C., *i.e.* the optimum for the disease, is scarcely practicable because the temperatures required are so high that they are difficult to maintain and are dangerous to the crop. The temperature should, therefore, be kept as much below 22° C. as is practicable. During March and April the average temperature is usually much below 22° C. and, although infection may occur if humid conditions obtain, the disease makes little progress. From May to August the temperature is favourable for leaf mould, and efforts should be made to keep the glasshouses cool by ample ventilation.

2. *Humidity.* Dry atmospheric conditions should be maintained continuously especially in summer when temperatures are so favourable to the disease. Experiments indicate that dry day conditions alone are of

little use and that humid nights may be largely responsible for the disease. During warm nights (average temperature 18° C.) the humidity should not exceed 75 per cent. During dull or rainy periods the ventilators should be left open to avoid a hot, humid atmosphere.

3. *Ventilation.* This reduces both the temperature and the humidity, and causes air movement. In spring, it should be given whenever weather conditions are favourable. As early as the season permits the ventilators, including doors and box ventilators, etc., should be left open continuously.

4. *Watering.* When possible watering should be done early and on fine days only. In dull weather it should be reduced to a minimum. Excessive watering produces soft, susceptible plants. Overhead watering is done frequently in spring, *i.e.* when it is difficult to ventilate freely, and is usually discontinued in summer, *i.e.* when full ventilation is possible. The reverse would probably give better results as far as leaf mould is concerned. If, however, growers deem the practice necessary in spring it should be done early on fine days only.

5. *Air movement.* This removes the layer of high humidity from the neighbourhood of the leaves. It is facilitated by opening the box ventilators and doors and by pruning of the lower foliage. Too close planting should be avoided.

Grateful acknowledgment is made to Dr W. F. Bewley for advice and criticism during the course of the work.

SUMMARY.

1. A study of the relation of atmospheric temperature and humidity to tomato leaf mould (*Cladosporium fulvum*) has been made with a view to assisting growers to control the disease by cultural methods.

2. The optimum temperature for the various stages of leaf mould is about 22° C. At 10° to 15° C. severe infection occurs under humid conditions, but the disease develops very slowly.

3. Humidities exceeding 90 per cent. are very favourable. At 22° C. infection is severe at 80 per cent. humidity but is rare at 70 per cent. At 18° C. infection and subsequent development are considerably retarded at 80 per cent. humidity.

4. Experiments indicate that seasonal variations in the severity of leaf mould are related to temperature.

5. Data are given which show that humid conditions obtain in large glasshouses for several hours each night and that these conditions are largely responsible for the severity of leaf mould.

6. Wetting of the foliage caused by overhead watering does not appear to favour the disease. Excessive watering produces turgid, susceptible plants.

7. A basis for controlling leaf mould by cultural methods is suggested.

(*Received June 5th*, 1929.)

CARBON DIOXIDE IN RELATION TO GLASS-HOUSE CROPS

PART IV. THE EFFECT ON TOMATOES OF AN ENRICHED ATMOSPHERE MAINTAINED BY MEANS OF A STOVE

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Introduction.

Experiments conducted at this station during 1924 and 1925 have shown that increased crops may be obtained by growing tomatoes and cucumbers in an atmosphere enriched with carbon dioxide (1). It was considered, however, that the chemical method then used for generating the gas was unsuitable for use on commercial nurseries and it was necessary to investigate other means of artificial enrichment.

During 1926 and 1927 attempts were made to obtain enriched atmospheres by means of a portable stove burning patent fuel, and also by fermentation of a mixture consisting of tomato haulms and stable manure.

The experiments were carried out in a glasshouse partitioned into six chambers, each having a capacity of approximately 2000 cubic feet and containing 56 tomato plants. The manurial treatment and other cultural operations were the same in all the chambers and conformed to the usual commercial practice.

As in former experiments samples of the atmosphere were taken by displacement, and analyses were made by means of the Haldane apparatus (2).

Experiments during 1926.

The experiments were designed:

1. To compare the effectiveness of atmosphere enriched by means of (*a*) a fermentation process, (*b*) a stove burning patent fuel, (*c*) the chemical method used in earlier investigations.

2. To ascertain whether, in the case of tomatoes grown in enriched atmospheres, it is more profitable to grow two crops each year.

3. To ascertain whether mechanical mixing of the carbon dioxide and air is essential as far as crop yield is concerned.

For the purposes of these objectives two successive crops were grown in 1926 and were treated as described below.

First crop. Each chamber was planted on January 15th with 56 tomato plants of an early variety, Tuckswood, which were "stopped" at the third truss and no side shoots allowed to develop. Treatment was given from March 3rd to May 15th as follows:

Chamber *X* 1. Carbon dioxide by fermentation of a mixture of tomato haulms and stable manure.

Chambers *X* 2 and *X* 4. Controls. Normal atmosphere.

Chamber *X* 3. A stove burning patent fuel was used from 9 to 11 a.m. and 3 to 5 p.m. each day, five blocks of fuel being used on each occasion.

Chamber *X* 5. An average concentration of 60 parts carbon dioxide per 10,000 of air from 9 to 10 a.m. daily, the gas being generated from sodium bicarbonate and concentrated sulphuric acid and distributed by a fan.

Chamber *X* 6. Treatment similar to that in *X* 5 except that no fan was used.

Table I.

The effect of atmospheres enriched with carbon dioxide upon crop yield in tomatoes (1926).

		Production					
		First crop			Second crop		
Chamber	Treatment	Lb. per plant	Tons per acre	Relative	Lb. per plant	Tons per acre	Relative
X 1	Carbon dioxide by fermentation (1st crop)	1·93	11·58	101	—	—	—
	Carbon dioxide by stove method from 9 to 11 a.m. and 3 to 5 p.m. daily (2nd crop)	—	—	—	2·35	14·10	112
X 3	Carbon dioxide by stove method from 9 to 11 a.m. and 3 to 5 p.m. daily	2·47	14·82	129	2·27	13·62	108
X 5	Carbon dioxide by chemical method; 60 parts of gas from 9 to 10 a.m. daily. Fan used	2·01	12·06	105	2·18	13·08	104
X 6	As in *X* 5 except no fan used	2·37	14·22	123	2·07	12·42	98
X 2	Control. Normal atmosphere	1·71	10·26	—	2·06	12·36	—
X 4	,, ,, ,,	2·13	12·78	—	2·13	12·78	—
	Av. of control chambers	1·92	11·52	100	2·10	12·60	100
	Av. of treated chambers (omit *X* 1)	2·28	13·68	119	—	—	—
	,, ,, ,, (*X* 1 and *X* 3)	—	—	—	2·31	12·86	110
	,, ,, ,, (*X* 5 and *X* 6)	—	—	—	2·12	12·75	102

Second crop. Each chamber was planted on June 7th with 56 tomato plants, variety Rochford's Improved Ailsa Craig, which were "stopped" at the fifth truss and no side shoots allowed to develop. The treatment, given from July 7th to August 27th, was similar to that for the first crop except that in X 1 the stove method was used instead of the fermentation process.

The results are summarised in Table I.

The effectiveness of the various methods used to obtain enriched atmospheres.

(*a*) *The fermentation process.* Reference to Table I shows that no increased yield was obtained by this process. This result was anticipated because the concentration of carbon dioxide was almost normal.

The fermenting material consisted of a mixture of three parts steamed tomato haulms and one part stable manure frequently moistened with a 1 per cent. solution of cane sugar. The mixture was placed in a tank of 10 cubic feet capacity inside the chamber. Tomato haulms are plentiful at the end of the season and if they could be made to yield sufficient carbon dioxide by fermentation, the cost of the process of obtaining enriched atmospheres would be lowered. The method, however, seemed unpractical and was not tested upon the second crop.

(*b*) *The stove method.* Of the methods used to obtain enriched atmospheres in 1926, the stove apparatus produced the greatest increase in crop yield. The increase was 29 per cent. in the first crop and was 10 per cent. in the second crop. The smaller increase in the latter crop was probably due to the shorter period of treatment and to a severe attack of tomato leaf mould (*Cladosporium fulvum*).

(*c*) *The chemical method.* This was used in chambers X 5 and X 6 and the only definite increase obtained was one of 25 per cent. in the first crop in X 6. It is difficult to understand why no increase occurred in X 5, because similar treatment increased the crop in former experiments.

The cultivation of two crops in one season.

In a previous paper (1) reasons were given for assuming that, in the case of tomatoes grown in enriched atmospheres, the cultivation of two successive crops may be more profitable than that of a single crop grown throughout the season. The total yield in X 3, *i.e.* the most productive chamber in 1926, was 4·74 lb. per plant. Thus, under the experimental conditions, the cultivation of two crops, planted January and June respectively, was not profitable. The result should not be taken as a guide

to commercial methods, however, because, owing to the coal strike, fuel became difficult to obtain, and the second crop came to maturity slowly and suffered from leaf mould which affected carbon dioxide assimilation.

The effect of mechanically mixing the carbon dioxide and air.

During the earlier investigations the carbon dioxide was mixed with the air of the chamber by means of a fan. Such a rapid distribution, while being desirable for experimental purposes, may be unnecessary as far as crop yield is concerned. The latter point was examined in 1926 as follows:

In each of the chambers *X* 5 and *X* 6, 6 lb. of sodium bicarbonate were decomposed by sulphuric acid and during decomposition the gas was distributed by means of a fan in *X* 5, while in *X* 6 it was allowed to diffuse into the atmosphere. The results, given in Table I, show that in the first crop increases of 5 and 25 per cent. were obtained in *X* 5 and *X* 6 respectively, and that in the second crop the yields were almost equal in the two chambers. The results indicate that mixing of the carbon dioxide and air is not essential from the standpoint of crop yield.

The distribution of the gas in these chambers is worth noting. Analyses of samples taken in *X* 5 are given below and show that the fan caused a rapid and almost uniform distribution of the gas.

Distribution of carbon dioxide in X 5 *immediately after decomposition.*

	Wall of chamber			
	North	South	East	West
Concentration* 6 in. from ground	97	91	91	94
,, 6 ft. ,,	98	101	93	88

* Throughout the paper the concentration of carbon dioxide is given in parts per 10,000 of air.

Analyses of samples taken in *X* 6 are given below and show that following upon a short period of marked stratification the gas became almost evenly distributed.

Distribution of carbon dioxide in X 6 *immediately after decomposition.*

	Wall of chamber			
	North	South	East	West
Concentration 6 in. from ground	659	450	451	626
,, 6 ft. ,,	39	75	47	31

Distribution of carbon dioxide in X 6 at various times.

	Time in minutes after decomposition			
	0	15	30	60
Concentration 6 in. from ground	491	86	63	34
„ 6 ft. „	44	82	67	29
„ 12 ft. „	55	101	68	40

Comparison of the stove and chemical methods used to obtain enriched atmospheres.

In the foregoing experiments daily treatment with the stove differed from that of the chemical method in several important respects. Chambers in which the stove was used received 35·3 cubic feet of gas daily which was generated continuously over two periods each of 2 hours, the concentration at each period being at least 18 parts per 10,000 for 1·5 hours as shown by the following data:

Time in minutes from commencement of treatment	0	30	45	60	105	135
Concentration	3·5	14·6	18·4	23·1	23·5	18·2

On the other hand, chambers treated by the chemical method received 25·68 cubic feet of carbon dioxide daily, all of which was generated in a few minutes, giving a high initial concentration which decreased gradually until the end of the hour when the ventilators were opened. The average concentration was approximately 60 parts per 10,000 of air for 1 hour as shown below.

Concentration immediately after decomposition	94
Concentration 1 hour after decomposition ...	32
Average for 1 hour	63

The better results obtained by the stove method may be attributed to any or all of the above mentioned differences.

The effect of atmospheres enriched by the stove method upon cucumbers.

This was tested in a cucumber house of 6,500 cubic feet capacity containing 72 plants, which were planted on February 23rd and grown in the usual commercial manner. Commencing on March 15th, 10 to 15 blocks of fuel were used twice daily from 9 to 11 a.m. and 3 to 5 p.m., but on July 3rd the experiment was discontinued owing to the unhealthy appearance of the crop. This deleterious effect was first noticed on April 30th and was possibly due to absence of ventilation; efforts to detect carbon monoxide in the atmosphere of the house were unsuccessful.

The experiment was interesting, however, because it showed that enriched atmospheres may be obtained in moderately large glasshouses. The concentration of carbon dioxide when ten blocks of fuel were used is given below, the stove being situated in the centre of the house and the samples taken in two positions, *A* and *B*, which were midway between the ends of the house and the stove.

(1) *With cucumber beds and plants absent.*

Time in minutes from commencement of treatment	0	45	75	95	120	150
Concentration. Position *A*	3·6	14·1	28·9	21·0	24·0	18·3
„ „ *B*	4·7	—	25·0	28·6	34·4	22·4

(2) *With cucumber beds and plants* (72) *present.*

Time in minutes from commencement of treatment	0	30	45	80	105	120	150
Concentration. Position *A*	16·3	30·8	43·6	51·1	47·9	46·7	37·1
„ „ *B*	23·8	32·4	44·9	44·1	43·6	44·8	33·0

The above data show that the maximum concentration was 34·4 parts per 10,000 of air when the beds and plants were absent and was 51·1 when these were present. The results indicated that the presence of the beds and plants was responsible for the higher concentration in the latter case, and suggested that the amount of carbon dioxide in ordinary cucumber houses during the season was much above the normal. This suggestion was borne out by analyses of samples taken in various houses. For example, the concentration in four different cucumber houses at 9 a.m. on April 29th was 20, 39, 26, and 29 parts per 10,000 respectively, while, as shown below, the minimum concentration during the day in one cucumber house on May 10th was 13 parts.

Time	9.0 a.m.	11.0 a.m.	12.0 p.m.	2.0 p.m.	4.30 p.m.	5.45 p.m.
Concentration	39·4	18·1	15·6	13·3	14·9	18·5

It may be that, under the weak light conditions prevailing in cucumber houses, the concentration of carbon dioxide, which is continually reinforced by decomposition of the stable manure in the cucumber bed, is sufficient for the requirements of the crop. Further work is necessary to test this assumption.

Experiments during 1927.

In view of the results obtained in 1926 a more extensive trial of the stove method of artificial enrichment was made in 1927. Since in former experiments the greater part of the increased crops obtained in the treated

chambers had been picked by the end of June, it was decided to compare the effect of treatment throughout the season with that of treatment for the first half of the season only. The experiments were, therefore, arranged as follows:

Chambers X 1 and X 4. Stove used from April 1st to September 30th.
,, X 3 and X 6. Stove used from April 1st to June 30th.
,, X 2 and X 5. Controls. Normal atmosphere.

Each chamber was planted on March 15th with 56 tomato plants, variety Balch's Ailsa Crag. The stove was used from 9 to 11 a.m. and 3 to 5 p.m. daily, five blocks of fuel being burned on each occasion giving a concentration of at least 18 parts carbon dioxide per 10,000 of air for 1·5 hours (see p. 85). The results, summarised in Table II, show that the average increase in crop was 16 per cent. where plants were treated throughout the season and was 9 per cent. where treatment was given for the first half of the season only. In the latter case the production

Table II.

The effect of atmospheres enriched with carbon dioxide upon crop yield in tomatoes (1927).

Chamber	Treatment	Production: Lb. per plant	Tons per acre	Relative
X 1	Stove used twice daily from April 1st to Sept. 30th	7·00	42·00	115
X 4	,, ,, ,, ,, ,,	7·13	42·78	117
X 3	Stove used twice daily from April 1st to June 30th	6·78	40·68	112
X 6	,, ,, ,, ,, ,,	6·41	38·46	106
X 2	Control. Normal atmosphere	6·09	36·54	—
X 5	,, ,, ,,	6·04	36·24	—
	Average of control chambers	6·06	36·39	100
	Average of treated chambers (X 1 and X 4)	7·06	42·36	116
	,, ,, ,, (X 3 and X 6)	6·59	39·54	109

Table III.

Showing the monthly production in the various chambers.

Chamber	Period of treatment	Production (lb. per plant): May	June	July	Aug.	Sept.	Oct.	Total
X 1	April 1st to Sept. 30th	0·01	2·14	1·32	1·62	1·07	0·84	7·00
X 4	,, ,,	0·05	2·56	1·47	1·20	1·15	0·70	7·13
X 3	April 1st to June 30th	0·04	2·51	1·58	1·03	1·05	0·57	6·78
X 6	,, ,,	0·01	2·05	1·81	1·13	0·81	0·60	6·41
X 2	Control chamber	0·05	2·34	1·03	1·13	0·89	0·65	6·09
X 5	,, ,,	0·04	2·26	1·31	1·09	0·81	0·53	6·04

during the second half of the season was scarcely above that of the control chambers. This is shown in Table III which gives the monthly pickings.

Thus, the results suggest that it is necessary to treat the plants throughout the season. It is worth noting that the summer of 1927 was abnormally wet and sunless and that an increase greater than 16 per cent. would possibly be obtained in a normal summer.

The stove treatment in relation to commercial practice.

Consistent increased yields were obtained in chambers where the stove was used in 1926 and 1927. These results lead to a consideration of two questions, namely, (1) whether the method is suitable for use on commercial nurseries, (2) whether the increase in crop is sufficient to warrant commercial exploitation of the process. With regard to the first question it is concluded that the method is very suitable for use under commercial conditions. To fill and light the stoves is very simple and they require no further attention. They are portable, well insulated, and may be placed among the plants. In the experiments described in this paper the nearest plants were 1 foot distant from the stove and only those leaves immediately above the stove were scorched. No smoke is produced. The amount of fuel supplied and hence the carbon dioxide may be varied in accordance with prevailing weather conditions.

Consideration of the second question shows that the 16 per cent. increase obtained under the experimental conditions is insufficient to cover the present cost of stoves and fuel. Hence, either a greater increase in crop or lower costs are necessary to make the method economically feasible.

Grateful acknowledgment is made to Dr W. F. Bewley for suggestions and advice during the course of the investigation.

SUMMARY.

1. The effect on the tomato crop of atmospheres enriched in respect of carbon dioxide liberated by two methods with possibilities of commercial application, (1) a fermentation process, and (2) a portable stove, is compared with that of carbon dioxide liberated by acid from sodium bicarbonate.

2. The concentration of carbon dioxide obtained from the fermentation process was insufficient to give any increase in yield. Using the stove method, which gave a concentration of at least 18 parts per 10,000

for 1·5 hours twice daily, the average increase in crop was 16 per cent. where plants were treated throughout the season and was 9 per cent. where they were treated for the first half of the season only.

3. Relatively high concentrations of carbon dioxide are shown to be normally present in cucumber houses. It is suggested that, under the weak light conditions prevailing in such houses, the concentration is sufficient for the requirements of the crop.

4. Mechanical mixing of the carbon dioxide and air is not essential from the standpoint of crop production.

5. In the case of tomatoes grown in enriched atmospheres, the cultivation of two crops, planted January and June respectively, was not profitable under the experimental conditions.

6. The stove method of treatment is discussed in relation to commercial practice.

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APPARATUS FOR THE GROWING OF PLANTS IN A CONTROLLED ENVIRONMENT

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(With Plates V and VI and 7 Text-figures.)

CONTENTS

INTRODUCTION.

The need for controlled environmental conditions in the study of the physiology of plants in health and disease has long been recognised. The expense involved in the construction of the apparatus necessary for this kind of work has, however, deterred workers from attempting such experiments. Little work of the kind appears to have been done in this country except for the researches of Gregory on the physiology of *Cucumis* (2,3), in which plants were grown under controlled conditions of air temperature, humidity and illumination. In the United States, however, a much more extensive development of this type of work, com-

mensurate with the greater availability of funds, has been achieved. The earlier work in that country was confined to experiments on the relation of soil temperature to certain diseases. The control apparatus for this purpose, developed at Wisconsin by L. R. Jones (7, 8) and his co-workers, has become the standard type known as the "Wisconsin soil temperature tank." Later workers, especially Peltier (11), Hottes (4), and Johnson (6), progressed further and constructed apparatus for controlling air conditions either simultaneously with or independently of soil factors. Facilities for such work reached their culmination in the controlled glasshouses at the Boyce Thompson Institute for Plant Research at Yonkers (1).

In connection with early experiments on the angular leaf spot disease of cotton the writer constructed a simple chamber in which air temperature and humidity were controlled and artificial light provided (12). The results of experiments in this apparatus led to an interest in the work being taken by the Empire Marketing Board, which provided a grant for the construction of the more elaborate tanks and chambers described in this paper. It is not proposed here to give details of experiments actually carried out in the apparatus but only to describe the construction and fitting up of the chambers. Later papers will deal with the experimental results of studies on the disease referred to above. Full constructional details with scale drawings are given to render the article as useful as possible, since it is understood that the construction of similar apparatus is being considered by other workers.

THE GENERAL PROBLEM OF CONTROL.

The environmental factors, any or all of which may be of importance in influencing the incidence, severity or spread of any particular disease, fall into three categories and may be tabulated as follows:

Biological		
Flora and fauna of the soil and air		
Chemical		
Soil nutrients	Air, composition	
Physical		
Soil temperature	Soil condition	Air movement
Soil moisture	Air temperature	Light intensity
Soil aeration	Air humidity (and rain)	Light quality
Soil reaction		

Of these, the first two groups may be considered as special subjects of investigation and outside the scope of the present inquiry. The apparatus to be described has been devised for the study of the physical

factors of the environment only. While the other factors, however, are omitted from the consideration of the effect of the physical environment, they must be known, or at least, if not known, kept constant. It is axiomatic that in studying the influence of any particular factor or factors the other conditions must remain unchanged or fluctuate similarly for all the experimental plants, but it is surprising how frequently this elementary principle is overlooked in such investigations. Results have been published, for example, on the relation of soil temperature to various diseases which affect not only the roots but also the aerial parts of plants, without information on the conditions environing those aerial parts, and comparisons have been made between the results of different experiments where it is at least unlikely that all other factors besides that under investigation are alike in the separate experiments. It is not maintained that information of value cannot be derived from studies of a single factor which is controlled while the others fluctuate, but only that comparisons between experiments where these unknown fluctuations are dissimilar may lead to erroneous conclusions. The recognition of this principle brings one to the greatest practical difficulty in the devising of apparatus for this work, the difficulty of *independent* control of the factors. Most of the physical factors are interrelated so that a change in one may produce a change in any or all of the others, and this resulting change may in some cases be large. For example, a small change in air temperature within a closed chamber produces a relatively large change in the atmospheric humidity measured as percentage relative saturation or as saturation deficit, although no change in the actual weight of water vapour present has been made. Now in the automatic control of physical factors the usual method consists in maintaining the required condition as near as possible to a mean value, the amplitude of the fluctuations on either side of the mean being determined by the sensitivity of the control apparatus. Since these fluctuations will affect the other factors a greater or less "strain" will be thrown on the apparatus controlling these factors and may result in a less accurate control. It follows therefore that the more accurately any particular factor can be controlled the easier will be the control of the others. It is unlikely that small variations in a factor will affect the disease under study and for this reason one would be tempted to use less sensitive apparatus, but for the reasons given control as exact as can reasonably be attained is necessary. This applies particularly to the control of temperature since the maintenance of a constant humidity is the most difficult problem and it is greatly facilitated by careful attention to the temperature control.

It is obvious that the external conditions will considerably affect the ease of control of the chamber. The ideal for very accurate work would be to instal the chambers in a room in which the atmospheric conditions were already roughly controlled, but the additional cost of this would be considerable. A compromise has been made at Rothamsted by installing the apparatus in a small room in which the door and window are at opposite ends. A small porthole type of exhaust fan is fitted to a louvre box ventilator in the window, the rest of which is darkened, and this is arranged to blow air continuously into the room, the door being left open to provide a through draught. Owing to the very considerable heat given off by the flood-lights it is usual to arrange for the lighting to be given only at night, so that the drop in outside temperature during the night is compensated by this heating effect. By this means the room temperature is kept usually within a 5–10° C. range. Good control of the air conditions within the chambers at any temperature above 20° C. and of the soil temperature above 13–15° C. can be obtained throughout the year except during very hot periods in the summer.

The particular practical difficulties of control will be dealt with in the separate considerations of the individual factors.

THE CONTROL APPARATUS.

General.

The apparatus consists of three main parts, shown in diagrammatic section in Fig. 1. These are the soil temperature tank (A, Fig. 1), the air chamber (L, Fig. 1) and the lighting apparatus (X, Fig. 1). These three parts are standardised and interchangeable between the six complete units. The dimensions and proportions given have been selected as suitable for the particular study (diseases of cotton) for which the apparatus was devised, but others might be more suitable for different conditions of work. The construction of the parts and method of control of the factors will be taken under the separate heads.

Soil temperature tank.

Tanks. The tanks used for the control of soil temperature follow the dimensions and principles of the "Wisconsin soil temperature tanks" but with considerable modification. The inner lining of the unit is a metal tank (B, Fig. 2), the walls of which are made of 22-gauge galvanised iron sheets and the bottom of 18-gauge sheets, the internal dimensions being 39 in. by 21 in. and 28 in. deep. The tank is made with a flange 3½ in. wide turned out at right angles to the sides to lap over the upper edges

of the outer wooden casing. This outer casing (*D*, Figs. 2 and 3) is of $\frac{7}{8}$ in. grooved and tongued deal boards and has outside dimensions of

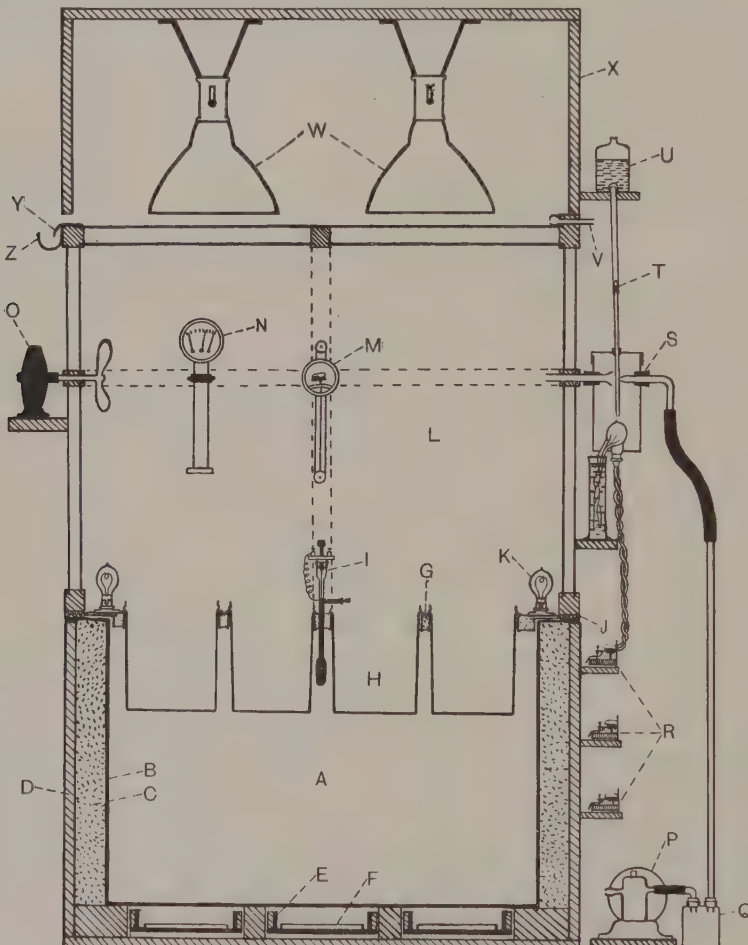

Fig. 1. General diagrammatic section of apparatus. *A*, soil temperature tank; *B*, galvanised iron lining; *C*, granulated cork insulating layer; *D*, outer wooden casing; *E*, drawer for heater; *F*, heating net; *G*, cover of tank; *H*, soil containers; *I*, thermostat for tank; *J*, felt strip; *K*, heating lamp for air chamber; *L*, air chamber; *M*, thermostat for chamber; *N*, hygrostat; *O*, fan; *P*, electric blower; *Q*, trap for oil; *R*, relays; *S*, humidifier; *T*, drip device; *U*, reservoir; *V*, inlet pipe for water screen; *W*, lamps; *X*, lamp gallery; *Y*, lead spout; *Z*, gutter.

47 in. by 29 in. and $32\frac{1}{2}$ in. deep, leaving a space of 3 in. between the walls and the metal tank and 4 in. between the bottoms. The tank rests

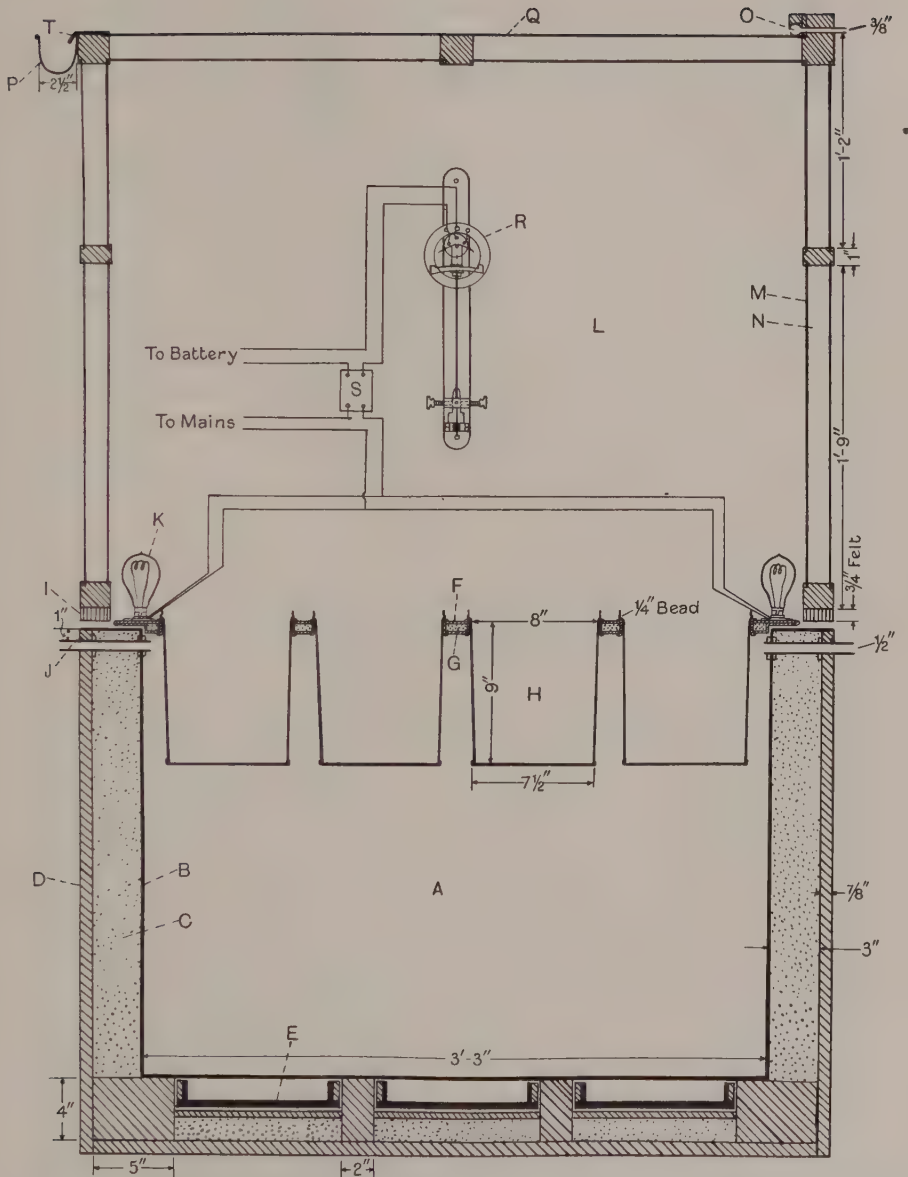

Fig. 2. Longitudinal vertical section of apparatus (to scale). *A*, soil temperature tank; *B*, galvanised iron lining; *C*, granulated cork insulating layer; *D*, outer wooden case; *E*, drawer for heater; *F*, metal cover; *G*, "Celotex" lining of cover; *H*, soil container; *I*, felt strip; *J*, inlet pipe to tank; *K*, heating lamp for air chamber; *L*, air chamber; *M*, glass wall; *N*, dead air space; *O*, inlet pipe for water-screen; *P*, gutter; *Q*, single upper sheet of glass; *R*, thermostat for chamber; *S*, relay; *T*, lead spout.

on three wooden bars 5 in. by 4 in. nailed to the side and back bottom corners of the casing and two transverse battens, 2 in. by 4 in., $10\frac{1}{2}$ in. apart on the floor of the casing. Between these battens a space of 2 in. from the bottom of the casing is enclosed by $\frac{1}{4}$ in. wooden boards. These spaces and the space between the walls of the casing and the tank are filled with granulated cork for heat insulation (*C*, Figs. 2 and 3). Shallow drawers (*E*, Figs. 2 and 3), to hold the heating units, slide in the remaining spaces below the bottom of the tank. These drawers are constructed of $\frac{1}{2}$ in. deal with a bottom of $\frac{1}{4}$ in. Uralite asbestos board, the sides being lined with the same material. The front 4 in. of the drawers are made of solid wood with a half-cut flange to fit a similar recess in the outer casing. The construction will be clear from Figs. 2 and 3. A short length of $\frac{1}{2}$ in. iron pipe at each end of the tank 1 in. from the top, passing through the tank and casing and held in place by back nuts provides for a flow of water through the tank.

Heaters. The heating units (*H*, Fig. 3) are electric resistance nets of wire woven on an asbestos framework, 18 in. by 8 in. made to specification by the Cressall Manufacturing Co., Eclipse Works, Birmingham. They can be made of any loading, but for temperatures up to 35° C. 150 watts loading each has been found suitable. They are laid loosely on two pieces of glass tubing cut to fit the drawers lengthwise. Lengths of rubber-insulated cable are soldered to the two ends of the resistance wire and these pass through holes bored in the solid wood front of the drawer and to the ends are attached porcelain-insulated one-way cable connectors. Further protection and insulation of these leads inside the drawers is provided by lengths of glass tubing slid over them. According to the temperature at which it is required to run the tanks the heating units may be wired in series (giving a total loading of 50 watts) or in parallel (450 watts) or in any combination. The advantage of this method of heating the tanks over the heater tube and lamp method used in the Wisconsin apparatus is that in the event of any failure the heaters are accessible with ease and without any dismantling of the apparatus. In addition, owing to the low temperature at which they run (below red heat) the nets have a practically unlimited length of life. Further, the distribution of heat is much more uniform than with the vertical heater tube used in the Wisconsin tanks.

Cover. The cover, shown in plan in Fig. 4 and in section in Figs. 2 and 3, consists of a top and bottom of 24-gauge tinned iron (*F*, Figs. 2 and 3) with a double layer of $\frac{1}{4}$ in. "Celotex" between (*G*, Fig. 2 and 3). The lower sheet of metal is bent twice at right angles over the edges of

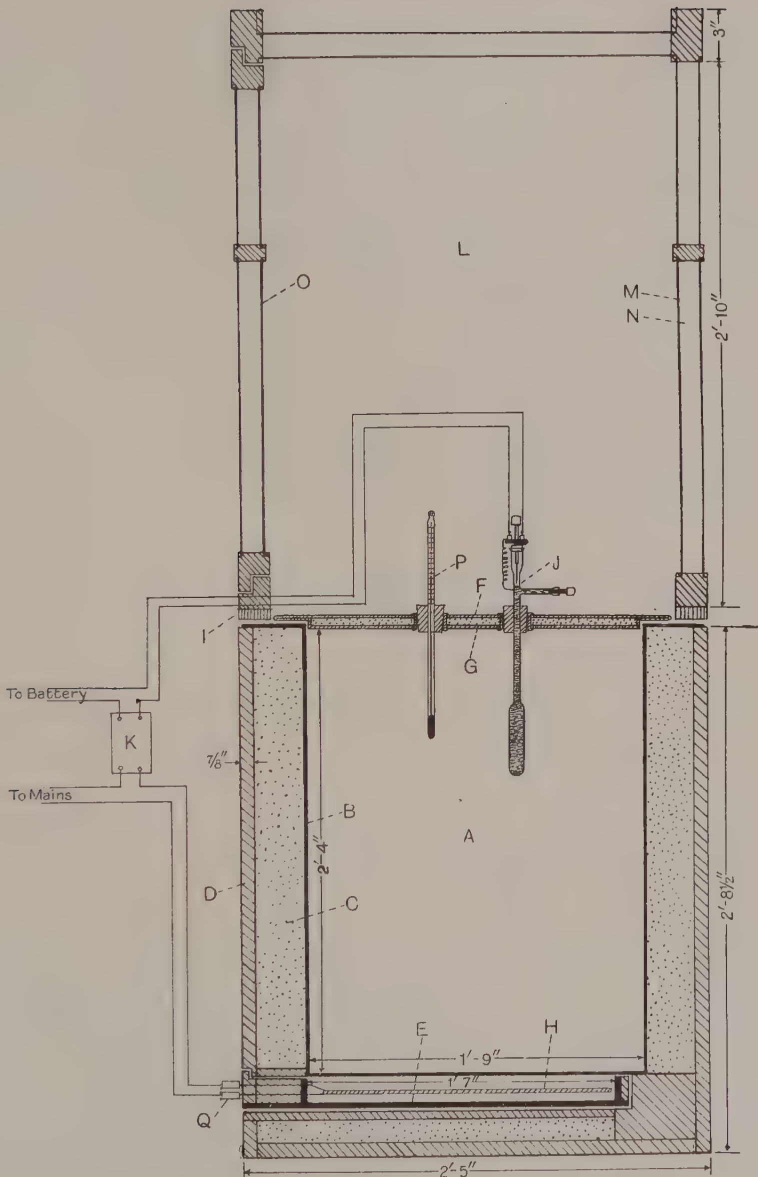

Fig. 3. Transverse vertical section of apparatus (to scale). *A*, soil temperature tank; *B*, galvanised iron lining; *C*, granulated cork; *D*, wooden casing; *E*, drawer for heater; *F*, metal cover; *G*, "Celotex" lining of cover; *H*, heating net; *I*, felt strip; *J*, thermostat for tank; *K*, relay; *L*, air chamber; *M*, glass walls; *N*, dead air space; *O*, door; *P*, thermometer for tank; *Q*, porcelain cable connectors.

the insulating material and soldered to the upper sheet to form a flange 2 in. wide which rests on the edges of the tank. The cover has eight 8 in. openings for the soil containers and two $1\frac{1}{2}$ in. holes for the thermostat and a thermometer. All the openings are lined with galvanised iron collars, fitted round the inside edges, bent over, and soldered watertight. The whole cover is given two coats of waterproof white paint. A cellulose paint such as "Luc" has proved most satisfactory.

Soil containers. The soil cans (*H*, Fig. 2) are made of 24-gauge tinned iron with internal side seams, well soldered so as to be watertight. They are 9 in. deep over all, 8 in. diameter at the top and $\frac{1}{2}$ in. less at the bottom so that they can be easily removed. A rolled bead or "swage" is made 1 in. from the top to support them in the openings in the cover. It should

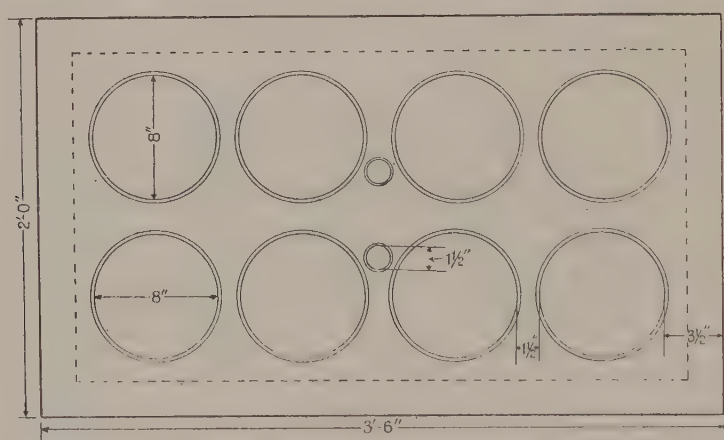

Fig. 4. Plan of cover (to scale).

be pointed out that this depth (effectively 8 in.) has been selected as convenient and suitable for the purpose in view, but deeper tins could be employed where necessary. The depth of the tank is arranged with this in view, although the chief advantage of so considerable a volume of water is in the greater steadiness of the temperature control and in the more uniform temperature distribution around the soil tins.

Thermostat. The thermostat (*J*, Fig. 3) employed is a modification of the standard electric mercury-toluol thermoregulator used for water baths. It consists of a mercury bulb about $4\frac{1}{2}$ in. long and $\frac{3}{4}$ in. diameter, sealed to a tube 8 in. in length with a bore of about $\frac{1}{8}$ in. carrying a side arm fitted with an adjusting screw. An adjustable nichrome needle passes through a cork in the widened end of the tube, and a piece of platinum wire sealed into the tube opposite the side arm provides the

other contact. The whole is filled with clean mercury; a drop of pure paraffin oil may be put on the top of the mercury to prevent oxidising. Control within $\frac{1}{4}$° C. or less is easily obtainable with this type of thermostat. The heating current is controlled through a relay (to be described later) operating from a 4-volt battery.

Other soil conditions. No attempt has been made as yet to control the other two factors which are not predetermined by the choice of the soil, namely, soil aeration and soil moisture. In the experiments on the disease under study it has been found satisfactory merely to supply water when inspection of the soil indicates the necessity.

AIR CHAMBER.

Construction. For the control of atmospheric conditions double-walled glass cases (*L*, Figs. 2 and 3) were built to fit on to the standardised soil temperature tanks, 3 ft. in height. The frame is constructed of 2 in.

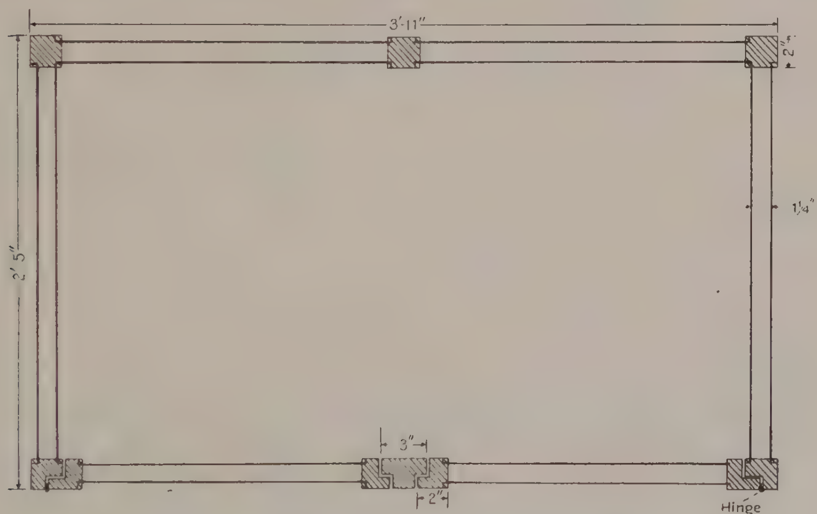

Fig. 5. Horizontal section of air chamber (to scale).

by 2 in. teak with 2 in. by 1 in. rails rather more than half-way up the side. Selected 21 oz. clear window glass is used for glazing, the panes being recessed $\frac{1}{4}$ in. and fixed with a $\frac{1}{4}$ in. corner bead. This leaves a dead-air space of $1\frac{1}{2}$ in. (*N*, Figs. 2 and 3) between the glass walls, providing some degree of heat insulation. Two doors, recessed with the frame (Figs. 3 and 5), occupying the whole of one side provide full access to the interior. The top of the frame is constructed with 2 in. by 3 in.

material with a 2 in. by 2 in. transverse rail. The inner roof consists of two sheets of glass like the walls, but the upper is a single sheet of 26 oz. glass resting in recesses in the side rails and supported in the middle by the transverse rail: $\frac{1}{4}$ in. strips nailed around the sides of the top rails while the putty used for fixing the top sheet is still soft provide a water-tight joint. The top of the case thus forms a shallow trough $1\frac{1}{2}$ in. deep. At one end is a $\frac{1}{2}$ in. "compo" gas-pipe (*O*, Fig. 2) with a connecting-tube of $\frac{3}{8}$ in. brass soldered to the middle, the latter passing through a hole in the middle of the wooden rail. This compo tube is perforated with small holes about 2 in. apart except in the middle where about six holes $\frac{1}{2}$ in. apart are made to provide a greater flow of water in the middle of the trough beneath the lights. At the other end the wooden side is cut

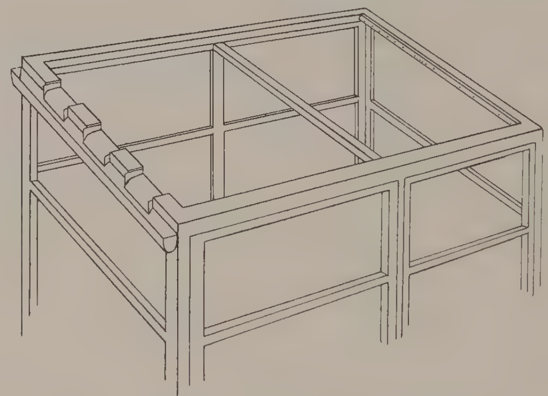

Fig. 6. Perspective view of top of air chamber.

away to leave three gaps $4\frac{1}{2}$ in. wide. The gaps are lined with lead sheet bent so as to form a lip to lead the water into a gutter of galvanised iron (*P*, Fig. 2) screwed on to the side of the chamber at a slight angle and provided with a down-pipe leading to the drain. The thickness of the lead, which just overlaps the glass, provides a slight obstacle to the flow of water, so that a water screen about $\frac{1}{8}$ in. deep is obtained. The even flow of the water is facilitated by having the whole apparatus slightly higher at the inflow end.

A thick layer of felt glued to the underside of the bottom rails of the cases provides a good seal with the soil temperature tank below.

The details of construction of the cases will be apparent from a study of Figs. 2, 3, 5 and 6.

Control of air temperature.

Heaters. The heat for the air chambers is provided by two carbon filament lamps held in batten holders screwed to the lid of the tank, a slot being cut with a hacksaw in the side of each holder to admit the flex for the electrical connection. The lamps are wired in parallel across the mains, with the relay, operated by the thermostat, in one main lead. The choice of lamp depends upon the temperature required within the chamber, the end aimed at being to obtain such a rate of heating that the lamps are on and off for approximately equal times. For temperatures above 25° C. during the winter 32 candle-power lamps are suitable. The direct radiant heat from the lamps is screened from the plants by cylinders of asbestos card pinched together at the top with a paper-clip and perforated with a number of holes.

Thermostat. The thermostat (*R*, Fig. 2) used for the control of the air chambers is one made by the firm of John Grundy, Ltd., City Road, London. This is a bi-metallic thermostat which may be adjusted to operate its two-way switch at any predetermined temperature from below freezing-point to 80° C. The contacts of the two-way switch are short platinum wires fused into a sealed glass tube about 1 in. long and $\frac{3}{8}$ in. diameter. This tube contains a globule of mercury and an inert gas. The tube is tilted from side to side (thus changing the electrical contact) by a pin projecting from the free end of the thermostatic strip and passing into a bifurcated member mounted on a pivoted steel shaft working in sapphire bearings, the whole arranged in a movable carriage. An important advantage of this thermostat is that should the apparatus not be in use, or should the temperature rise or fall from the point at which the instrument is set, the thermostatic strip and pin is free to move and pass right out of the bifurcated member in either direction; thus the strip is never held, and no strain is put upon it which would obviously upset its adjustment.

As used in the air chambers the thermostat acts as a single-pole switch operating the tilting-tube relay to be described; two only of the three contacts in the tube are therefore used. A control of air temperature within a range of $\frac{1}{2}$° C. is easily obtained.

Control of air humidity.

The automatic control of humidity presents a greater problem than that of temperature. Considerations of space and of expense prohibited the instalment of the various somewhat elaborate types of air-conditioning

apparatus used at Wisconsin (6), Illinois (4), the Boyce Thompson Institute (1) and other American institutions. Earlier experiments (13) had led to the development of a simple form of humidity control which had been found to give satisfactory results in small chambers, and this has been used with modifications for these larger cases.

The humidifier. The apparatus (Fig. 7) depends for its action on the controlled vaporisation of water from a wet muslin surface. A tin (*A*, Fig. 7), some 8 in. in height and 4 in. in diameter (the familar "Bath Oliver" biscuit tin is very suitable), has a hole made in the bottom somewhat larger than the diameter of the metal neck of an 8 candle-power carbon filament lamp which passes through the hole and is held in place by a piece of wide rubber tubing serving as a washer. The bulb of the lamp is closely covered with one end of a strip of muslin stitched in place, the other end passing through a smaller hole in the bottom of the tin and dipping into a vessel of distilled water. A hole towards one side of the lid is provided with a rubber stopper and through this passes a constant-drip device to ensure continuous wetting of the muslin. This device consists of a piece of glass tubing drawn out to a fine point and sealed into a wider tube at the point where the tapering begins. The fine tip is protected from blocking by a short sealed tube small enough to slide within the wide tube. Both the wide tube and the cap having been filled with filtered distilled water, the latter is carefully slid down the tube until it covers the fine tip. The whole is connected to a reservoir of distilled water supported on a shelf screwed to the top of the chamber. A constant supply of water then drips slowly from the lower tube, the end of which is drawn out to a blunt taper and reaches nearly to the top of the lamp. The rate of drip is controlled by the size of the fine tip, and should be fast enough to ensure that the muslin is always wet. A rate of 14 or 15 drops per minute is satisfactory for average conditions.

Near the top of the tin are bored two holes diametrically opposite, each having a rubber stopper through which passes a short length of glass tubing. The tube to serve as inlet is drawn out to a wide "jet" and the outlet to the chamber is made somewhat funnel-shaped. The "jet" of the inlet is usually placed about $\frac{3}{4}$ in. from the funnel of the outlet, and is connected to an electric blower[1] *via* a Wolff's bottle which acts as a trap for any oil blown over by the motor and also as a divider for the air supply, since one blower can supply two or three chambers. The lamp in the tin is controlled through a relay by a hair hygrostat within the chamber. When the lamp is not glowing, the air stream from the "jet"

[1] Lennox No. 1 Blower, supplied by Messrs A. Gallenkamp and Co.

passes straight through to the chamber without taking up any appreciable amount of moisture in its passage through the tin, and this entry of air, which is usually drier than that within the chamber, results in a lowering of the relative humidity within the latter. At the required point

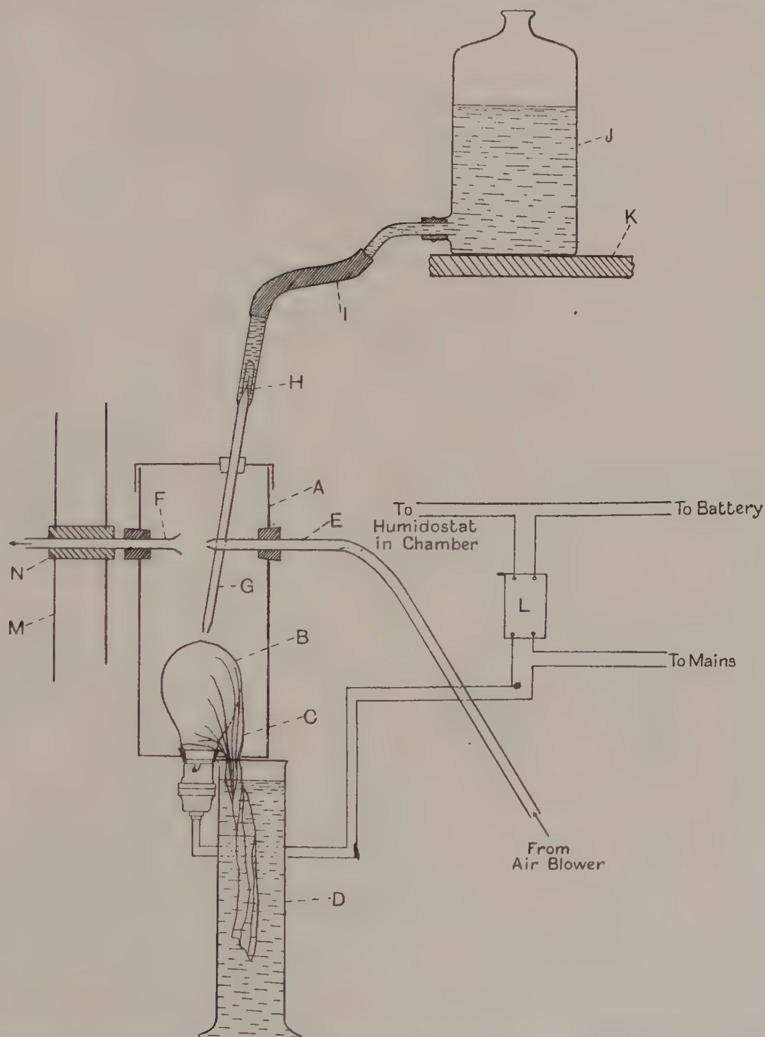

Fig. 7. Details of humidifier. *A*, tin; *B*, 8 candle-power carbon filament lamp; *C*, muslin covering lamp loosely; *D*, cylinder for water; *E*, air inlet to tin; *F*, air exit to chamber; *G*, tube to lead drip to lamp; *H*, drip jet; *I*, connecting tube to reservoir; *J*, water reservoir; *K*, bracket screwed to top of chamber; *L*, relay; *M*, glass walls of chamber; *N*, side rail of chamber.

the hygrostat releases the relay, switching on the current to the lamp. The heat rapidly vaporises water from the muslin and some of the vapour is carried into the chamber by the air stream until the making of the hygrostat contacts again switches off the lamp.

For low humidities it is necessary to dry the air before passing it through the apparatus, preferably by freezing. Additional control of the range of variation can be obtained by adjusting the distance between the inlet and outlet tubes within the tin. If they are placed wide apart the air stream will take up some water vapour on its passage through the tin, even when the lamp is off, while when close together little or none is carried through except when the lamp is glowing. For larger chambers, or high humidities at low temperature, it may be necessary to substitute a 16 candle-power or even a 32 candle-power lamp for the 8 candle-power one specified, but in this case an adequate supply of water must be ensured, since if the muslin becomes dry it will char, and the decomposition products are very deleterious to plants. An 8 candle-power lamp will not char the muslin appreciably even if the latter becomes dry.

The hygrostat. The hygrostat employed in these chambers for the control of atmospheric humidity is a direct reading hair hygrometer[1] provided with adjustable platinum-tipped contacts. Unfortunately, a hair hygrometer is not very constant in action and requires frequent checking and resetting. It is probable that a more satisfactory type of hygrometer could be devised for the purpose.

The hygrostat is checked periodically against a dew-point hygrometer placed within the chamber, and the necessary adjustments made.

Fans. Stratification of the air within the chamber with resulting temperature and humidity gradients is prevented by a small fan running continuously. It is necessary that the motors of these fans (as of the blowers) should be of the induction type, as series-commutator motors give constant trouble with fouled commutators and burnt-out brushes. Those used[2] are of the semi-enclosed "squirrel cage" induction type, of 1/50 H.P. at 1400 R.P.M., with 5 in. shaft extension and 6 in. diameter three-way blades. The motors are mounted on brackets of 1 in. deal screwed to the side of the chamber, the shaft extension passing through a hole bored in the side rail of the cabinet. Beyond occasional oiling these require no attention.

Relays. The relays used are of the tilting-tube mercury-break type[3]. A small solenoid with soft iron core is wired in series with a 4-volt battery

[1] Supplied by Messrs Negretti and Zambra.
[2] Supplied by Messrs The General Electric Co.
[3] Supplied by Messrs A. Gallenkamp and Co.

and the control instrument. Activation of the electro-magnet results in the attraction of the short arm of a bent lever, the other arm of which tilts up a small glass tube mounted on a platform provided with bearings. The tube contains a pool of mercury which, when the tube is horizontal, makes contact between two wires fused into the ends of the tube, and connected in series with the mains and the heating unit. When the tube is tilted by the lever the pool of mercury breaks, switching off the current. The relay takes approximately 0·05 amp. at 4 volts.

The relays for the six tanks and chambers, eighteen in all, are run off a single 30 amp.-hour 4-volt battery permanently connected to a trickle charger, the output of which is adjusted to balance the current taken by the relays.

The lighting apparatus.

In order to obtain a constant illumination which shall be the same for all the chambers, artificial light only is used. This is provided by two 500-watt gas-filled Osram lamps in special "Gecoray" flood-light reflectors[1]. The lamps are very lightly frosted over the lower half of the bulb to give a more even distribution of light. The reflectors are carried in special holders screwed to a wooden frame which stands on the top of the air chambers and is held from shifting by dowel pins fitting into holes in the wooden rails of the top. The reflectors are less than 1 in. from the water screen flowing over the top of the chamber. With these lights an average illumination of 800–900 foot-candles at the level of the cover of the tank is given. The main leads for the lamps are taken through a 50 amp. Venner time switch which can be set to give any required period of illumination. As stated above, where non-continuous illumination is used the dark period is usually arranged to be during the warm part of the day.

Summary.

An account is given of the construction of tanks and chambers for the growing of plants under independently controlled soil and air conditions. The plants are grown in soil tins sunk in a water tank, the temperature of which is automatically controlled. Double-walled glass chambers fit over the tanks, and within these the conditions of temperature and humidity are independently controlled. Artificial illumination is provided by two floodlights over each chamber, with 500-watt gas-filled lamps in each providing an illumination of the order of 1000 foot-candles. The chambers were erected under a grant from the Empire Marketing Board for research on the bacterial disease of cotton.

[1] Supplied by Messrs The General Electric Co.

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EXPLANATION OF PLATES V AND VI

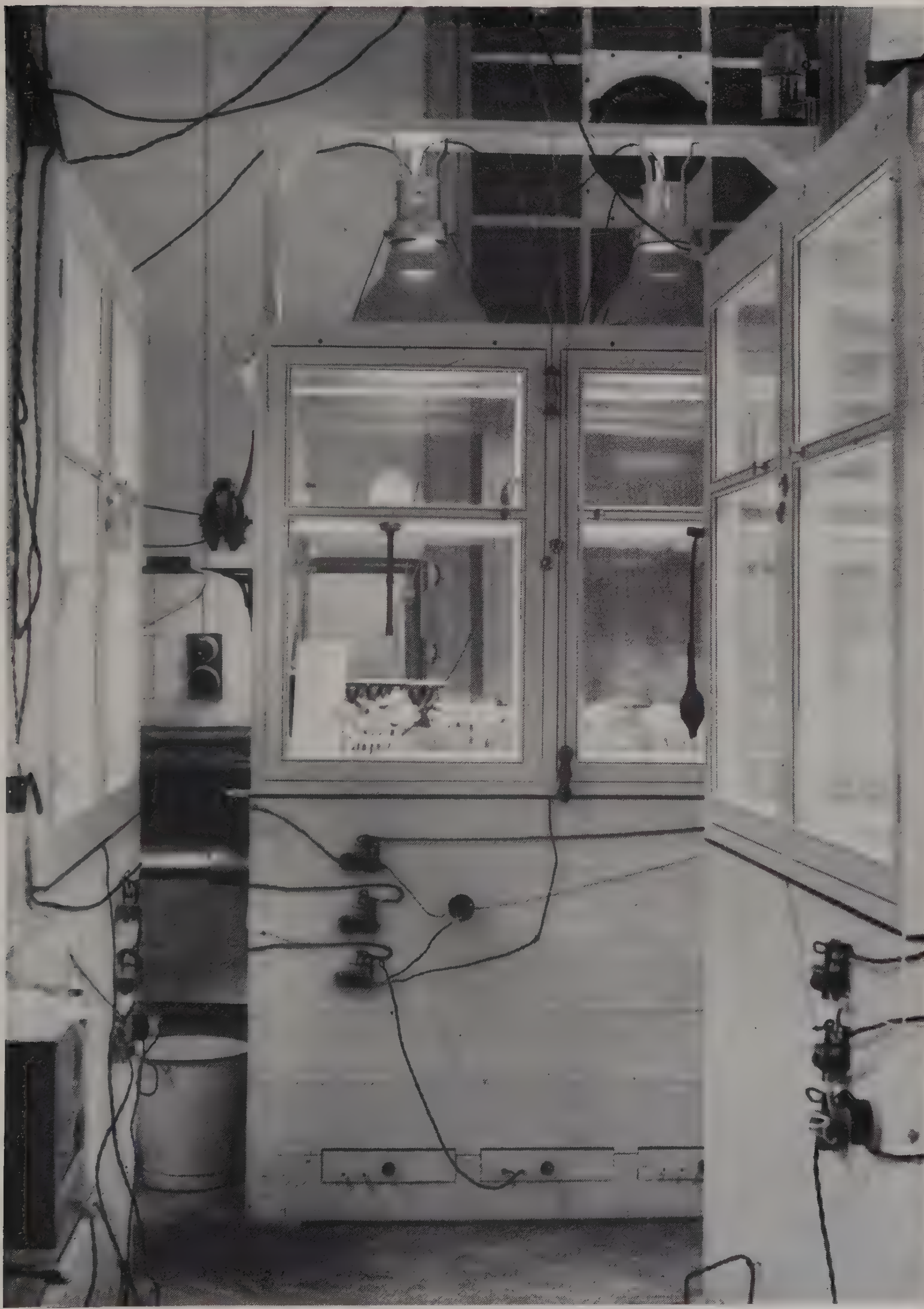
PLATE V.

General view of apparatus showing three of the chambers containing cotton seedlings.

PLATE VI.

View of single chamber. (N.B. The thermostat in the chamber is not of the type finally adopted.)

(*Received June* 16*th*, 1929.)



STOUGHTON.—Apparatus for the Growing of Plants in a Controlled Environment (pp. 90–106).

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A BOTANICAL STUDY OF HAY PLOTS

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Introduction.

While engaged in advisory work at Seale-Hayne Agricultural College, Devonshire, grassland problems were frequent. The results of the trial plots throughout the county at times were rather unexpected, and the reason not always obvious. It was with a view to testing the competitive relations of grasses and clovers, under south-western conditions, which led to the laying down of a few more plots for close observation and to trace the changes which occurred from year to year. The fact that Red Clover seed is not always true Late Flowering or Broad-leaved was the first important point gained. The failure of Alsike Clover was another gain, while the composition of the hay crop from year to year threw a flood of light on what had occurred and was still happening in some districts (5).

Unfortunately, it was not known that the Welsh Plant Breeding Station were engaged on somewhat similar problems until their publication appeared. Otherwise references would have been made to the excellent work just published from Aberystwyth.

The plots.

The plots were of the "door mat" type, 2 yards by 2 yards, and were prepared in 1921–2 from part of an old permanent pasture. The soil was ploughed and hand dug and the small 6-inch strips between the pairs of plots trodden hard and kept free from weeds. Between one pair of plots and another ran strips of 1 foot also trodden hard and kept free from weeds. During the last year of investigation these strips were hoed, permitted to grow, and closely cut; the turf forming very quickly. The soil consisted of a medium loam inclined to be slightly silty but not deficient in lime. The exposure was S.S.E. and the altitude 350 to 400 feet above sea-level. The average rainfall was about 40 inches. The plots were regarded from the outset rather as a feeler and to get botanical information before attempting any large-scale experiments on a chess-board principle.

The experiment.

The series of plots were put down simultaneously with others in order to discover (1) the competitive relationship of certain plants to each other under south-western conditions; (2) what proportion each plant gave towards the production of hay; (3) the final result of continuous cutting and removal of hay, without the addition of any manures, for several years.

The plots of Red Clover and Timothy, Cocksfoot and Alsike Clover, Perennial Rye Grass and Alsike Clover were to test the possibility of these mixtures for agricultural purposes in the South-west of England. Some were obvious mixtures, such as Red Clovers and Rye Grasses, except that more definite knowledge of the yield given by each was required. The Rye Grass and Wild White Clover, and Rye Grass and ordinary White Clover were experimental in order to discover what proportion of each was present after a few years and how the plants reacted under hay conditions. Many of the plots were not expected to yield outstanding results, but the history of the changes could be observed and noted. The plots of all legumes and all grasses respectively were of a purely experimental nature, in order to watch the struggle for existence and the changes towards a more stable vegetation under hay conditions.

The weights of seeds sown were based on the weight required to cover the ground with vegetation, expressed as per acre.

The size of the plots naturally introduces a considerable experimental error. Not only is this due to actual size and shape, but light could enter laterally on one side and to a certain extent on three sides. This was realised at the beginning of the experiment, but questions of cost, labour (as many other plots had to be controlled simultaneously), ease of comparison, and of making observations, outweighed other considerations. The weights have been left as pounds and ounces (16/13), and not converted to weight per acre so as not to magnify any error. The weights are not considered as such, but merely as a rough basis of comparison.

Methods adopted.

The plots were cut twice per annum except the first year when they were cut in the autumn. At first they were weighed green and also when dried. Owing to the difficulty of drying without leaving the material on the small plots and consequently upsetting the growth beneath, and also at times the unfavourable weather conditions, this method was abandoned. Drying the bulk indoors was also impossible owing to the diffi-

culty of carrying all without some loss and the huge space which would have been necessary with this and other work of a similar nature running concurrently. Repeated attempts were made to get some relationship between the green and the dry weight, both in bulk and for the separate species. After many efforts the results were not sufficiently stable to rely on any figure. For grasses the dry weight was $\frac{7}{22}$ of the green weight. Clovers and legumes were about the same, sometimes a little less according to circumstances. The weights recorded are the weights of the crop cut green.

Samples were carefully drawn from each plot and air dried in the laboratory. This, of course, introduces another difficulty as it was impossible to do the analysis by weight when green. In spite of this the difference in each case is sufficiently well marked not to make it unreliable for a general assessment of the proportion of each plant present in the hay. It was noted, while trying to find some factor for estimating dry from green weight, that the dry weight did not alter the order of the plots as regards yield, and that there was not any serious discrepancy between the proportionate yields of the plots, although there were some small variations.

The whole of each plot sample was carefully analysed out and placed in a marked package. When the series was completed they were weighed on the same day so as to keep the weights in proportion.

The weight of the first cut, 1927, was not taken, as it was then intended to leave the plots under pasture conditions. Owing to the writer leaving in October, however, the aftermath of 1927 was weighed and sampled so as to round off the experiment and bring it to a close.

Results.

1924. (Tables I and II.) At the end of the period of growth 1924, the weight of yield for 1923 being added, resulted as might be expected in the all-legume plot, 15 *a*, giving the highest yield. After this plot, the Red Clover plots, 9 *b*, 9 *a*, 13 *b*, 8 *a*, 10 *a*, 13 *a*, 10 *b*, and 11 *b*, showed very little difference and can be roughly placed in the second place. Such differences as do exist may be considered as experimental error. Plot 12 *a* gave a smaller yield than 9 *a* and 9 *b*. This is largely due to the different proportion of ingredients upsetting the balance. The Cocksfoot and Alsike plots were not a success, for 8 *a* and 16 *a* showed little difference. It will be seen that the Perennial Rye and Meadow Fescue Grass was not a successful combination, while Perennial Rye and Alsike, and Perennial Rye and Wild White Clover were poor in top growth. Unfortunately,

Seed sown in plots expressed as pounds per acre.

	8 *a*	8 *b*	9 *a*	9 *b*	10 *a*	10 *b*	11 *a*	11 *b*	12 *a*	12 *b*	13 *a*	13 *b*	14 *a*	14 *b*	15 *a*	15 *b*	16 *a*
Late Red Clover	10	—	10	—	10	—	—	12	12	—	10	—	—	—	6	—	—
Broad Red Clover	—	—	—	10	—	10	—	—	—	—	—	10	—	—	6	—	—
Wild White Clover	—	—	—	—	—	—	2	—	—	—	2	2	—	—	1½	—	—
White Clover	—	—	—	—	—	—	—	—	—	—	—	—	—	7	1½	—	—
Alsike	—	5	—	—	—	—	—	7	—	—	—	—	7	—	2½	—	7
Black Medick (*Medicago lupulina*)	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1¼	—	—
Birds Foot Trefoil	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—
Kidney Vetch	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1¾	—	—
Lucerne	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1½	—	—
Sainfoin	—	—	—	—	—	—	—	—	—	—	—	—	—	—	3	—	—
Perennial Rye	—	—	10	10	—	—	20	—	10	20	—	—	10	15	—	3¾	—
Italian Rye	—	—	—	—	10	10	—	—	—	—	—	—	—	—	—	3¼	—
Cocksfoot	—	18	—	—	—	—	—	—	—	—	—	—	—	—	—	1¾	7
Timothy	10	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1½	—
Meadow Fescue	—	—	—	—	—	—	—	—	—	20	—	—	—	—	—	3¾	—
Tall Fescue	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	3¼	—
Tall Oat	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	3½	—
Golden Oat	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2½	—
Rough S. Meadow	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—
Smooth S. Meadow	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	¾	—
Crested Dogstail	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1½	—
Meadow Foxtail	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1¼	—
Fiorin	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—
Sweet Vernal	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1½	—
Hard Fescue	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2½	—
Sheep's Fescue	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1¼	—

the Broad Red Clover proved to be impure, containing Late Flowering Red Clover plants. This fact led to a separate series of trials (3).

1925. (Tables I and III.) In this year the Timothy and Red Clover (Late Flowering) took the lead in yield, Timothy giving 75 per cent. of the yield from the first cut. Plots 9 *a* and 15 *a* show little difference. Plot 15 *a* consisted of Red Clover and Sainfoin, as far as legumes were concerned, a few of the other ingredients being present in small quantity but grasses gave a fair yield towards the total. Plot 13 *b* was interesting as Perennial Rye had crept in, doubtless due to seeds lying in the soil from the original pasture (1). Plot 11 *b* showed the presence of both Perennial and Italian Rye, the latter must have been from seeds outside the plot. Plots 9 *a*, 15 *a*, 13 *b* and 11 *b* tend to form a group occupying second place. Plot 13 *a* is interesting, since a considerable amount of Perennial Rye appeared. This is probably largely due to the presence of Wild White Clover in the plot, which although not adding directly to the plot does so indirectly by favouring Perennial Rye Grass (11). In 9 *b* the proportions are very similar to 1924. The same is true of 12 *a*. Plots 13 *a*, 9 *b* and 12 *a* form the third group. In 10 *b* the Italian Rye Grass has increased considerably since the previous year and Red Clover decreased. The same is true of 10 *a*, the two plots showing little difference. In 8 *b* the Cocksfoot has got the upper hand and Alsike, owing to the summer drought, has fallen far behind. In 15 *b* Tall Oat is the chief ingredient, although there were traces of Timothy, Cocksfoot, Sweet Vernal, Crested Dogstail, and Golden Oat. In 11 *a* Perennial Rye naturally gave the yield, although some other plants had crept in. In 16 *a* Cocksfoot practically overwhelmed the few remaining plants of Alsike Clover, though not so completely as in 8 *b*. Perennial Rye topped the Alsike in 14 *b* but the Clover was present. In 12 *b* Perennial Rye has kept Meadow Fescue in check, but is not so plentiful as it was in 1924. In 14 *a* Perennial Rye has kept the Alsike Clover in check and Yellow Suckling Clover has competed with it, the result being that the yield is small.

1926. (Tables I and IV.) Plot 10 *b* gave the highest yield but the quality was poor considering that 24 per cent. was Soft Brome, 21 per cent. Dock, and 18 per cent. Yorkshire Fog. The failure of the Red Clover and the dying out of the Italian Rye Grass allowed these plants to get things too much their own way. In 9 *b* Red Clover lasted well and with the Rye Grass gave a good yield of good quality. In 15 *a* the legumes came next. In 13 *b* the Wild White Clover having stimulated dormant seeds of Perennial Rye, the yield of Rye is 40 per cent. of the total, an

increase of 23 per cent. on the previous year. In 10 *a* the Red Clover has increased, and as might be expected Italian Rye has dropped to 20 per cent. The increase in Red Clover is due to the small proportion of Italian Rye in the total bulk. Plot 12 *b* presents a curious state of affairs. Rye Grass has fallen to 10 per cent., but the rest is made up of such interlopers as Black Medick 27 per cent., Bent[1] 29 per cent., and Rough Stalked Meadow Grass 13 per cent., as well as a few others. The bulk of the yield therefore consists of other plants than those sown. Plot 8 *a* gave a smaller yield in 1926, the fall being due not to the Timothy but to the failure of the Red Clover. In a depleted yield Timothy gave the same proportion of 75 per cent. In 14 *b* Bent and Yellow Suckling Clover gave 59 per cent. of the total. White Clover has died out, and as a result Perennial Rye has fallen to 11 per cent. from 82 per cent. In 11 *a* Wild White Clover has increased in the hay from 6 per cent. to 17 per cent., but Perennial Rye has fallen from 77 per cent. to 43 per cent. Yellow Suckling Clover has crept in and formed 17 per cent. of the total. This has helped to depress the Perennial Rye. Plot 11 *b* shows a drop in the percentage of Red Clover, a slight increase in the percentage of Italian Rye, and a large increase in Rough Stalked Meadow Grass, 8 per cent. Yellow Suckling Clover and 10 per cent. Soft Brome. In this plot, as elsewhere, it is evident that grasses soon crept into plots sown only with Clovers. Plot 15 *b* consisted chiefly of Tall Oat, Golden Oat, with traces of Cocksfoot, Fescues, Crested Dogstail and some Sweet Vernal. In 9 *a* the percentage remains much the same. Red Clover is about the same and there is a slight decrease in Rye, but a marked increase in Rough Stalked Meadow Grass. Plot 14 *a* is practically the only plot where Alsike Clover is present in any quantity and actually increases from 20 per cent. to 35 per cent. Rye has decreased from 52 per cent. to 19 per cent., while Rough Stalked Meadow Grass and Yellow Suckling Clover add much to the total yield. The high percentage of Alsike is due to some extent to the decrease in Rye Grass, but largely to the shelter of the Rye Grass in the early stages of the Alsike Clover. The chief bulk of plot 13 *a* is due to Bent, which rose from 4 per cent. to the high figure of 40 per cent. Rough Stalked Meadow Grass has increased but Rye has decreased, as has Red Clover. Plot 12 *a* shows an increase in Clover but a decrease in Rye Grass, Rough Stalked Meadow Grass has increased, while Bent has begun to creep into the plot. Plot 8 *b* Alsike Clover has made some appearance, but Cocksfoot has decreased, chiefly due to Bent which has increased from 4 per cent. to 22 per cent. In 16 *a* Cocksfoot has decreased from

[1] Bent = chiefly *Agrostis alba* var. *stolonifera* plus a little *Agrostis vulgaris.*

75 per cent. to 24 per cent., Alsike Clover has increased, but Yellow Suckling Clover has increased from 9 per cent. to 49 per cent. This large increase in Yellow Suckling Clover has had a smothering effect on the plot.

1927. (Tables I and V.) By the spring of 1927 all the plots were rather overgrown with interlopers. It was therefore decided to cease cutting for hay and cut to keep the growth in check, and, provided the grasses became dominant, to cut frequently to represent grazing. This plan was not followed out as the writer left the district at the end of October. In order to finish the experiment from a hay point of view, the autumn cut or aftermath was carefully made and analysed. Owing to an unfortunate accident the samples from 8 *a*, 8 *b*, 9 *a*, and 9 *b* were damaged and no analysis could be made. The yields were obtained and these with the analysis of the other plots were added to the previous results as a final observation.

The general result in 1927 was that all the Red Clover had practically disappeared. Wild White Clover was still present and holding the ground remarkably well in spite of fierce competition. Grasses and weeds were now dominating the situation, and certainly from a hay point of view gave the yield. In 13 *b* Perennial Rye, which had crept in, provided the yield. In Plots 12 *a* and 12 *b*, Rye again provided the yield. In 13 *a* Bent and Rough Stalked Meadow Grass supply the hay. In 10 *a* all the Italian Rye had disappeared, and much of the Clover, so that other grasses gave the yield with a little Red Clover. Plot 15 *a*, the all-legume plot, gave the poorest yield. There was a little Sainfoin and Red Clover with traces of Black Medick, Yellow Suckling Clover, Wild White Clover, and Birds Foot Trefoil. Grasses, chiefly Bent, gave 60 per cent. of the cut.

Discussion.

Yield. (Table I.) The actual weight of material from the various plots is not always a good indication of success or failure. Indeed, in many cases, especially in later years, it may be entirely misleading. It is not mere bulk that is the important point, quality always tells. Towards the end of the present experiment the weight of cut obtained from many, if not most of the plots, consisted of weeds (grasses), much inferior plants and weeds, and generally plants which were not those which came from the seeds orginally sown. In fact by the year 1927 many of the plots consisted of a totally different vegetation to that originally obtained. The composition of the various yields in October 1927 revealed the fact that most of the plots were reverting to a semi-natural vegetation

consisting of poorer types of grasses and many weeds (14). For instance, in 15 *a* by 1927 inferior grasses accounted for 60 per cent. of the yield. Even in 15 *b*, consisting entirely of grasses, by 1927 a large part of the yield was made up of the inferior and coarser grasses.

Another point of importance is that the actual percentage of ingredients may be much more flattering on paper than in actual fact. In 8 *b* the yield has been consistently low. Consequently the percentage proportion of weight yielded by Cocksfoot is flattering, unless one keeps constantly in mind that it is merely a high proportion of a rather poor yield. Plot 16 *a* is another example of the same thing. Both plots were Cocksfoot and Alsike Clover, but in different proportions. Unfortunately, as was feared, the drought spells proved too much for the Alsike Clover, and what might otherwise have been a very successful combination is not suitable for a dry district or even for a district liable to suffer from summer drought. There are, however, some parts of the south-west where Alsike Clover does well. Plot 14 *a* is another interesting case, for the actual percentage is flattering on paper as the yield has invariably been low. The higher percentage of Alsike Clover (14 *a*) in 1924, 1925 and 1926 is due to the fact that Perennial Rye gave more shelter by its early growth and tended also to conserve soil moisture better than Cocksfoot with its more open growth. Even in spite of this the yield was low, as Alsike would not give a very large proportion to the weight. The early spring drought of 1927 practically wiped out the Alsike Clover.

Plot 11 *a* was interesting, since the yield depended on the Perennial Rye Grass, as Wild White Clover would add little directly to the cut. The yield, though low, was steadily maintained. In 1926 there was actually 17 per cent. of Wild White Clover in the cut. By 1927 the tall growth was telling on the Wild White Clover, and other grasses and weeds competed fiercely with it, and, as a consequence, Perennial Rye fell to 11 per cent. It shows how well Perennial Rye and Wild White Clover agree, and that they make a splendid combination for a pasture (12). Plot 14 *b* is another interesting case where Perennial Rye and White or Dutch Clover were grown together, and having a certain resemblance to 11 *a*. The White Clover soon died, but some Wild White Clover appeared in its place. In this plot Yellow Suckling Clover and Bent seriously interfered with the two plants originally sown.

Plots 9 *a*, 9 *b*, 10 *a*, and 10 *b*, also 12 *a*, all came under one group. As far as the total yields are concerned there is little between 9 *a* and 9 *b* (69/13 and 69/5), while between 10 *a* and 10 *b* the difference is not large (61/7 and 64/7). In the case of 12 *a* it is 54. In the last case the pro-

portions sown meant greater competition. Owing to the fact that Broad Red Clover was not true to type it is not possible to make a very reliable comparison. It will be seen that in 1925 plot 9 *a* gave a higher yield than the others but fell below the others in 1926, while in 1927 it rose again to a fairly high position. Unfortunately there is no percentage figure in the 1927 yield of these two plots. Late Flowering Red Clover, however, played a large part in the yield, such as it was. A noticeable feature is the duration of Italian Rye Grass when cut early (11). This has been noted in other places where constant grazing prevented seeding. A few seeds may have been accidentally introduced into the plots, but this does not explain the high proportion of Italian Rye, as cutting took place before seeds were near maturity and usually at flowering time. So that here it is not a case of self-seeding. Plots 13 *a* and 13 *b* are interesting, since both yield and percentage proportions show strange differences. Till 1924 there was little difference, but, by 1925, plot 13 *b* began to pull ahead. Plot 13 *b* showed a little more Red Clover and 13 *a* a greater quantity of Perennial Rye Grass which had doubtless arisen in association with the Wild White Clover. Rough Stalked Meadow Grass and Bent began to show in both plots. In 1926 plot 13 *b* was still further ahead than 13 *a*, in spite of the fact that 13 *a* had more Red Clover and 13 *b* more Rye Grass. The crux of the whole matter was that 13 *b* had only 21 per cent. of Bent while 13 *a* had 40 per cent. By 1927 plot 13 *a* had little Red Clover, while 13 *b* had 33 per cent. in spite of a poor yield. There were traces of Wild White Clover in the hay of both plots. The grasses, chiefly Bent, Yorkshire Fog, and Soft Brome, amounted to 73 per cent. in 13 *a* and 37 per cent. in 13 *b*, the weeds being 9 per cent. and 14 per cent. respectively. The plot with Late Flowering Red Clover showed a superiority throughout in spite of the fact that there was some Late Flowering in the Broad Red Clover plot. Plot 12 *a*, though more heavily seeded, gave a smaller yield than either 13 *a* or 13 *b*. In 11 *b* Alsike gave nothing towards the cut, but it did compete with the Red Clover and consequently reduced the yield. The reduction was considerable the first year, not so much in the second, 1925, but in 1926, owing to the disappearance of Alsike, grasses and weeds crept in and reduced the yield by competing with the Red Clover.

In the plot of grasses 15 *b*, except in the first year when the Rye Grasses yielded 63 per cent. between them, the yield steadily decreased, not merely in weight but in quality. This was chiefly due to the smothering activities of Tall Oat. As it decreased open spaces occurred which soon filled with Bent, although Golden Oat and Yellow Suckling Clover

filled some of the gaps. By 1927 Bent (Fiorin included) had invaded the whole plot with the result that only Tall Oat, Tall Fescue (18), and some Black Medick made any appearance. Except in 1927, plot 15 *b* gave a poor yield compared with the others, and in that year most of the other plots had only grasses left. Plot 12 *b*, as was anticipated, was a failure. Fescue never made any real headway against the Perennial Rye (6). But for the Rye the plot would have been a mass of weeds. Even in 1926 Bent and Black Medick gave 50 per cent. of the yield, and by 1927 grass weeds had reached the large total of 96 per cent. of the cut.

Plot 11 *a* is interesting, as only Perennial Rye Grass gives weight to the yield, the Wild White Clover adding little directly, but aiding indirectly. The yield throughout was low but of good quality as the percentage figures indicate. Even Wild White Clover pulled up by the top growth gave towards the yield. Plot 14 *b* affords some comparison with 15 lb. of Perennial Rye Grass and 7 lb. of White Clover seeds sown. The yield is greater at first but by 1926 the yield was chiefly interlopers, such as Yellow Suckling Clover and Bent. By 1927 no less than 72 per cent. consisted of grasses. For this reason 14 *b* cannot be closely compared with 11 *a* owing to the difference in quantity of seeds sown. Wild White Clover began to appear in the later stages after the White Clover had died. The percentage of Rye Grass present in the two plots does not greatly differ for 11 *a* ended with 11 per cent. and 14 *b* with 12 per cent.

The most successful plot both as regards yield and quality was 8 *a*. Both Timothy and Late Flowering Red Clover held the ground right up to the end of 1926. By 1927 the Red Clover was dying out and the wet summer and autumn gave weeds and other grasses a splendid opportunity to compete with the Timothy Grass. Even in spite of this serious competition, Timothy held its own remarkably well and there was no marked diminution in the number of plants present (10). From a hay point of view and yield this was an extremely successful plot.

Competition. (Tables II, III, IV and V.) The problem of competition between the various plants is at times rather difficult of interpretation owing to the fact that some plants are shortlived (*e.g.* Italian Rye and Commercial Perennial Rye Grasses). When such plants die, gaps are left in the vegetation which are filled by weeds, inferior or useful grasses, and legumes. Such plants are interlopers, since they are not sown in the seed mixture and not desirable in the crop. Practically all agricultural soils contain seed of interlopers. Some of these interlopers may otherwise be very useful plants (*e.g.* Perennial Rye Grass and Wild White Clover). The success of a crop depends on the ability of the plants sown to agree with

each other, and to hold the ground against interlopers. In this article any plants other than those sown in the seed mixture for the plots are considered interlopers whether useful or otherwise. The interlopers with two exceptions came from seeds already in the soil before the hay plot seed mixtures were sown. The two exceptions were Italian Rye Grass and *Crepis taraxacifolia* (4).

As has been found elsewhere, Italian Rye Grass competes far more fiercely with Red Clovers than Perennial Rye Grass (8). The struggle is more intense between Italian Rye and Broad than with Late Flowering Red Clover. The more open growth of Late Flowering Red Clover means less shading of the rye grasses so that, as with Italian so with Perennial Rye, the best results are obtained with Late Flowering Red Clover.

Wild White Clover and Perennial Rye make a splendid combination and instead of there being competition, these two plants seem to favour each other. In many plots where Wild White or Perennial Rye was present the other appeared. The presence of the one seemed to stimulate the other (10). Generally Wild White Clover prepares the way, a point which is frequently noted in the improvement of a pasture (9). White or Dutch Clover and Perennial Rye are not so successful. As soon as the Dutch White Clover begins to die, weeds and other grasses creep in and the deterioration of the quality is rapid. Generally White or Dutch Clover is taller and can be pulled up by top growth, but its failure to last and hold the ground is a serious disadvantage. Plot 14 *a* had a heavier seeding than 11 *a*, yet the percentage of Perennial Rye in the final stage showed little difference. Plot 11 *a* had 11 per cent. and 14 *b* had 12 per cent. It is pretty evident from this that, as occurred on other plots, the difference in the quantity of seed sown—if not great—makes less difference than the amount of interference and competition caused by interlopers. In the case of 14 *b* interlopers accounted for no less than 72 per cent. of the yield; so that any real comparison between 11 *a* and 14 *b* is not possible. The ability of Wild White Clover to hold the ground well more than compensated for the heavier seeding of 14 *b*. The interlopers are chiefly Bent, Yorkshire Fog, and Soft Brome among grasses, Yellow Suckling Clover and Black Medick among legumes. All these plants are more or less indigenous to the soil from which the plots were made. In addition to these, Perennial Rye Grass and Wild White Clover also appear where circumstances are favourable. The fact that the last two appeared and dominated the paths during the last year showed that the seeds were in the soil. The Perennial Rye which came from seed in the soil was of

the true perennial type. In fact, had it been possible to finally graze the Rye hay plots and give reasonable treatment, the same vegetation which originally existed might finally have dominated the situation.

Perennial Rye Grass completely crushed out Meadow Fescue in 12 *b* (15). Grasses and weeds filled the gap and finally Perennial Rye was itself crowded out.

Perennial Rye and Alsike Clover agree extremely well. The open growth of Alsike Clover gives Perennial Rye full scope. Another point is that Perennial Rye shelters Alsike in the early parts of the year and tends to conserve soil moisture. The yield of 14 *a* was light but the proportion given by each of the ingredients was steady. Even when Perennial Rye was disappearing Alsike Clover still held its own.

Cocksfoot and Alsike Clover also agree well and there is no reason to suppose that under conditions more favourable to Alsike this plot would have given a very different result. The tufted and non-spreading growth of Cocksfoot affords too little shelter to Alsike in its early growth. Alsike Clover got a very bad series of checks owing to early drought and following on this the tall growth of the Cocksfoot rather shaded the Clover. It is a mixture which has done extremely well in some places, but where there is a liability to drought it cannot be recommended. Perhaps a still heavier seeding of Cocksfoot might improve matters.

Red Clover and White Clover do not combine too well (17). Naturally the taller growth of both Late Flowering and Broad Leaved Red Clover gives Wild White and White Dutch Clover little chance. Broad Red seems to be a fiercer competitor than Late Flowering Red unless the latter is laid by wind and rain. Another point is that Wild White Clover does better under these conditions of competition than White or Dutch. Plots 13 *a* and 13 *b* show that not only was Late Flowering Red Clover the better of the two in yield but in actual balance. By 1927 plot 13 *b* had 21 per cent. Bent, but 13 *a* had 40 per cent. The more open growth of Late Flowering helps the Wild White more than the denser foliage of Broad Leaved Red. As a result of this, after cutting, the greater carpet of Wild White Clover in the Late Flowering Red plot conserved the moisture better; moreover, weeds got less chance to creep in and Bent was better held in check. Another significant point was that there was more Perennial Rye in 13 *b* than 13 *a*, a sure sign of superiority. Another point of interest is that 13 *b* did better than 12 *a* (which had a heavier seeding of Late Flowering Red Clover and 10 lb. of Perennial Rye) as far as yield was concerned. Even allowing for the competition between the Rye Grass and the Red Clover it shows that Wild White Clover has

a real indirect value, for it holds the ground against weeds and tends to conserve moisture when the top growth is cut (11).

Red Clover and Alsike Clover, as was expected, did not prove a very satisfactory combination (12). Alsike does slightly better with Late Flowering than with Broad Red Clover. The fact that Late Flowering Red Clover is slightly later in maturing and its habit of growth more open gives Alsike Clover a better chance. In both cases, however, the Alsike made little progress and weeds crept in and finally competed with Red Clover itself.

Timothy and Red Clover did remarkably well. The tall erect growth of the Timothy did not interfere with the Red Clover and the stimulus of the Red Clover aids Timothy, which makes rather heavy demands for nitrogen. The result is that the two do not compete but are a splendid combination. From a practical point of view Timothy does not grow much aftermath, but if cut fairly early, Timothy and Red Clover together give a very fair aftermath. Another point is that until the Red Clover begins to disappear weeds get little chance to make headway and the ground remains remarkably clean. The question as to whether the 10 : 10 ratio is the best is still rather doubtful.

In the all-grass plot, 15 *b*, after the first year Tall Oat dominated the situation till 1927, when Tall Fescue, and a little Meadow Fescue, made an appearance for the first time (18). Delayed germination of the seed of of this has been noted frequently (15). Italian Rye is about the only grass which can compete successfully with Tall Oat, for Perennial Rye is not nearly so effective. That is, of course, when Tall Oat is seeded fairly heavy. The chief point about this plot is that Tall Oat dominated the plot and completely upset "the balance of power." The ultimate result was that inferior grasses got the dominating position. Bent, Soft Brome, and Yorkshire Fog had successfully invaded the plot in 1927. In spite of appearance the yield was never high, bringing out a point noted elsewhere that Tall Oat gives the impression of weight which is not justified by fact (7). The persistence of the Fescues is important and suggests that with little or no Rye Grass in the mixture they may prove useful (7).

The all-legume plot, 15 *a*, was entirely an experimental one. Naturally, at first Red Clovers dominated the situation, but as time went on the staying power of Sainfoin became evident. Another point is that it withstood a considerable amount of shading the first year and yet survived. Lucerne, as was expected, could not compete against the other legumes. Even Crimson Clover made little headway in the first year. As the Red Clovers died out, however, grasses invaded the plot and soon were

able to compete very successfully with the remaining legumes. Sainfoin was the only outstanding success of this plot, after the Red Clovers died.

Changes in the vegetation. (Tables II, III, IV and V.) The field for which the plots were prepared in 1921 was a good permanent pasture with a deep medium loam. It was not deficient in lime and must have been treated with phosphate previously although there was no definite record of this. The field was used as a pasture and grazed with sheep, cattle and horses. The vegetation was good, consisting largely of Perennial Rye Grass, Wild White Clover, some Cocksfoot, some Meadow Grasses (almost entirely Rough Stalked), a little Bent, Yorkshire Fog, and Soft Brome. The chief weeds, and they were not plentiful, were *Cnicus arvensis*, a few *C. lancolatus*, *Bellis perennis*, *Rumex obtusifolius et R.* spp. near the gate entrance. There were also present in some quantity Black Medick and Yellow Suckling Clover.

Latterly, the field has been laid up for hay after each spring grazing. The difference so far marked was that Soft Brome was more evident and to a lesser extent Yorkshire Fog, but Bent was not plentiful. Perennial Rye Grass and Wild White Clover still remained dominant, but Cocksfoot was more evident. Thistles also were more noticeable since they got an opportunity to grow up.

When the part of the field which now forms a forest nursery and botanical plots was broken up the ground quickly showed the presence of a varied weed flora. The chief weeds were Charlock and Poppy. Bent was also prevalent and *Lychnis dioica et diurna*, *Convolvulus arvensis*, *Capsella Bursa-pastoris*, *Cerastium* spp. and *Senecio vulgaris* were in certain years extremely common. In the case of Convolvulus it was present to a slight extent in the field, and during periods of drought the flower of Convolvulus is frequent in parched pastures in the district. The seeds of the others had doubtless been present to some extent and also the fact that hay is often fed to stock in winter in the field means the introduction of the seeds of many common weeds. Another point of some importance is that Oats used for feeding stock contain sometimes a very appreciable amount of the seeds of Charlock. This and the facts brought out by Miss Brenchley in 1918 (2) explain the presence of all these weeds in pasture land. In Devon, as elsewhere, the varied number of weeds which grow on the banks of the hedges usually succeed in shedding their seeds before the hedges and banks are cut and trimmed. The only plants which wandered into the hay plots which were not native to the soil were *Crepis taraxacifolia* (4) and Italian Rye Grass. The seeds were introduced either by wind or by people visiting the plots. Italian Rye appeared in some

of the plots as an interloper in 1925 and 1926, but had disappeared by 1927. *Crepis taraxacifolia* did not appear till 1925, but continued after that year and was still present in 1927.

Other weeds present during the time the hay plots were under investigation were: *Ranunculus repens et parviflorus*, *Sagina procumbens*, *Anagallis arvensis*, *Stellaria media*, *Sherardia arvensis*, *Veronica arvensis*, *V. hederafolia et agrestis*, *Prunella vulgaris*, *Myosotis arvensis*, *Taraxacum officinale*, *Hieraceum* spp., *Sonchus oleraceus*, *Plantago lanceolata*, *Poa annua*. None of these weeds was present in any quantity, but merely a few plants scattered throughout the plots.

Agrostis spp., *Bromus mollis*, *Holcus lanatus*, *Poa trivialis*, *Medicago lupulina* and *Trifolium minus* were the chief interlopers in the plots. In 1925 Rough Stalked Meadow Grass and Bent had successfully obtained a footing in many of the plots (Table III). This was still more evident in 1926 (Table IV), while Yorkshire Fog and Soft Brome had also made their appearance in quantity. By 1927 (Table V) Bent, Yorkshire Fog, Soft Brome and weeds had dominated most of the plots. A glance at the figures in Table V shows that in most of the plots over half the weight of hay was composed of plants which must be ranked as weeds.

The appearance of many of these plants in the hay plots is therefore not surprising. The most interesting point, however, is that Bent (present to a slight extent in original vegetation) should not merely invade but tend to dominate some of the plots. Soft Brome was naturally favoured by the constant cutting. It shows very clearly the importance of attention to grassland, either hay or pasture or both (2, 14). The field which was hayed and grazed showed no resemblance to these hay plots in 1927. In many of the plots Bent covered from 50 to 75 per cent. of the surface of the ground. The poor top growth and the impoverishment of the soil gave Bent a splendid opportunity. Cutting in place of grazing also favoured Soft Brome and Yorkshire Fog, so that both increased steadily. In fact the general trend of vegetative change in this direction is exactly in keeping with many of the grasslands of the south-west (5). It explains the history, or much of the history, of many meadows which were little better and some worse than the hay plots in 1927. The only difference is that in one case the history of the "artificial" bad farming is known, whereas in actual practice the history can often only be obtained by conjecture and a close scrutiny of the plants present. In a few cases information is available and exactly bears out in practice what has been achieved by artificial means, although some of these meadows are occasionally grazed. An initial difference probably is that the original seeds mixture, where

one was sown, will have been a very bad or poor one, and that the vegetation reverted to a semi-natural type almost from the start. Even in the case of plots 15 *a* and 10 *b*, by 1927 there was not much difference between the final vegetation of the plots. As far as the changes in the vegetation of the hay plots in the earlier years are concerned, there is nothing very outstanding except the well-known fact that plants which grow well together and do not compete too severely with each other make a more stable vegetation. Given reasonable care, those plants sown may hold the ground against interlopers for a reasonable, in some cases an indefinite time, and the same is true of pasture conditions. The Wild White Clover and Perennial Rye Grass combination is one of the latter class, and Timothy and Red Clover are of the former. Climatic conditions may of course alter general possibilities such as the case of Cocksfoot and Alsike Clover. The greater success of Perennial Rye and Alsike Clover is a case of the former sheltering the latter in the early stages of its growth, and also Perennial Rye spreading over any bare spaces and reducing drought effect. Where, however, conditions are against one species and it fails, the other species suffers and may be overwhelmed. This becomes, of course, still more evident when several plants are involved instead of only two. In such a case the balance is more delicate and the failure of any one may upset the whole artificial association (11). It once again brings out the fact that good management plays quite as large, if not a larger part in maintaining the balance of useful plants than either the original mixture or the natural vegetation from which the present vegetation may have been derived. There is no doubt that bad management can reduce all to the same common denominator of poverty (13, 16).

Conclusions.

Red Clovers give the greater part of the hay yield when present with another grass, clover, or a mixture of these.

As soon as the Red Clovers decrease, weeds and inferior grasses appear and rapidly gain a dominant position. The surviving grasses of those originally sown cannot hold weeds and inferior grasses in check. A complete grass mixture with tall and rapid growing species present can hold the ground against interlopers for a considerable time (15 *b*).

Timothy and Late Flowering Red Clover make a splendid combination for hay. With a persistent Late Red (Cornish Marl or Montgomery Red) the yield could be maintained for years.

Alsike Clover is not successful where summer drought occurs. It grows better with Perennial Rye Grass than Cocksfoot.

Perennial Rye Grass and Wild White Clover form a natural association when biotic or other factors keep the herbage short.

Red Clovers and Alsike Clover do not agree. The same antagonism exists between Rye and Fescue grasses.

Tall Oat Grass is not suitable for a hay mixture under ordinary conditions, as it tends to upset the balance of the mixture.

Sainfoin is very persistent and, like Cocksfoot, a good drought resister.

Tall Fescue Grass, which formed the bulk of the Fescue yield, although slow in development is very persistent and a good drought resister. This grass deserves further trial.

A pasture vegetation (*e.g.* Perennial Rye and Wild White Clover) is not suitable for hay and can only yield a light crop. The continued use of this for hay alone upsets the association of grass and clover, and thus encourages inferior grasses and weeds.

The previous vegetation may, by means of buried seeds, have a considerable effect on the subsequent vegetation raised from sown seeds.

The changes in the vegetation of the poorer plots follows the retrogression of many hay fields under certain farming conditions.

The slow but steady introduction of arable weed seeds into grassland by various means is a lurking menace during the early seedling stages of any sown seeds mixture.

The final state of many of the plots bore a striking resemblance to many poor grasslands in the south-west of England. Continuous cutting and removal of hay with no manuring impoverishes the soil, weakens the competitive capacity of the useful grasses and clovers, and encourages Bent, Yorkshire Fog, Soft Brome and weeds.

By 1927 Bent, Soft Brome and Yorkshire Fog, with a smaller but varying proportion of weeds, dominated most of the plots.

The fluctuating proportions of Bent, Yorkshire Fog and Soft Brome from year to year show that competition with other species is the chief factor in deciding the proportions of these grasses present in the plots.

The seeds mixture used must not only be suitable for the purpose required, but also suitable to the soil and climatic conditions of the district. If unsuitable the crop fails to form an artificial association strong enough to keep out undesirable species (often native to the soil) which will ultimately dominate the sown plants.

Sound management is the most important factor in the successful handling of grassland for hay, since it virtually means the controlling of competition between the plants present in such a way as to favour the useful and discourage the less nutritious plants and weeds.

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Table I.

Weight of hay from plots 1923–7 *in pounds and ounces.*

(18/8 = 18 pounds, 8 ozs.) Plots 2 × 2 yds.

	1923	1924		1923–4	1925			1926			1927	1923–7
Plot	Oct.	June	Aug.	Total	June	Nov.	Total	June	Oct.	Total		Total
8 *a*	/9	18/8	7/11	*26/12*	24/4	3/9	*27/13*	8/7	1/6	*9/13*	8/12	*73/2*
8 *b*	/9	8/0	6/7	*15/0*	11/4	3/15	*15/3*	3/10	1/6	*5/0*	9/12	*44/15*
9 *a*	/14	19/0	7/2	*27/0*	20/1	5/3	*25/4*	3/9	4/12	*8/5*	9/4	*69/13*
9 *b*	/14	21/8	5/11	*28/1*	16/4	4/3	*20/7*	7/11	5/6	*13/1*	7/12	*69/5*
10 *a*	1/0	18/0	7/12	*26/12*	12/15	4/3	*17/2*	8/15	2/2	*11/1*	6/8	*61/7*
10 *b*	/14	18/0	6/13	*25/11*	12/8	4/15	*17/7*	10/1	4/0	*14/1*	7/4	*64/7*
11 *a*	/9	5/8	2/4	*8/5*	9/12	3/15	*13/11*	8/1	1/0	*9/1*	9/4	*40/7*
11 *b*	/10	21/8	3/5	*25/7*	17/8	5/10	*23/6*	6/1	2/14	*8/15*	10/12	*68/8*
12 *a*	/11	15/0	5/0	*20/11*	16/2	3/7	*19/9*	3/2	2/2	*5/4*	8/8	*54/0*
12 *b*	/4	4/8	2/5	*8/1*	6/11	3/13	*10/8*	5/12	5/0	*10/12*	9/4	*38/9*
13 *a*	/13	21/0	4/10	*26/7*	17/2	4/5	*21/7*	4/5	2/10	*6/15*	8/0	*62/13*
13 *b*	/11	22/0	4/5	*27/0*	18/7	5/10	*24/1*	8/1	3/12	*11/13*	8/12	*71/10*
14 *a*	/11	7/8	3/12	*11/5*	6/11	2/3	*8/14*	4/15	2/2	*6/17*	7/0	*34/4*
14 *b*	/7	9/0	7/13	*17/4*	8/2	3/7	*11/9*	4/12	4/14	*9/10*	8/6	*46/13*
15 *a*	1/9	27/0	4/10	*32/9*	17/5	7/5	*24/10*	5/2	6/14	*12/0*	6/0	*74/0*
15 *b*	/15	8/8	4/5	*13/12*	12/3	2/5	*14/8*	5/13	2/12	*8/9*	9/8	*46/5*

Table II. 1924.

Percentage by weight of plants present in hay.

	8 *a*	8 *b*	9 *a*	9 *b*	10 *a*	10 *b*	11 *a*	11 *b*	12 *a*	12 *b*	13 *a*	13 *b*	14 *a*	14 *b*	15 *a*	15 *b*	16 *a*
Late Red Clover	42	1	59	—	—	42	—	93	54	—	—	93	—	—	24	—	—
Broad Red Clover	—	—	—	37	34	—	—	—	—	—	97	—	—	—	51	—	—
White Clover	—	—	—	—	—	—	1	—	—	—	—	—	—	8	—	—	—
Alsike	—	17	—	—	—	—	—	2	2	—	—	—	32	—	—	—	33
Yellow Suckling	—	1	—	—	—	—	2	2	—	2	—	—	1	—	—	—	2
Black Medick	—	—	—	—	—	—	—	—	—	—	—	—	—	—	14	—	—
Sainfoin	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	—	—
Perennial Rye	—	—	40	60	—	—	93	—	41	85	—	—	65	87	—	21	—
Italian Rye	—	—	—	—	64	58	—	—	—	—	—	—	—	—	—	43	—
Cocksfoot	—	78	—	—	—	—	—	—	—	—	—	—	—	—	—	3	60
Timothy	57	—	—	—	—	—	—	—	—	—	—	—	—	—	—	3	—
R. S. Meadow Grass	—	—	—	—	—	—	—	3	—	—	1	4	—	1	1	—	1
Fescues	—	—	—	—	—	—	—	—	—	9	—	—	—	—	—	1	—
Tall Oat	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	29	—
Bent	—	2	—	—	—	—	—	—	—	—	2	—	—	—	—	—	—
Weeds	1	1	1	3	2	—	4	—	3	4	—	3	2	4	1	—	4
Lucerne	—	—	—	—	—	—	—	—	—	—	—	—	—	—	7	—	—

Table III. 1925.

Percentage by weight of plants present in hay.

	8 *a*	8 *b*	9 *a*	9 *b*	10 *a*	10 *b*	11 *a*	11 *b*	12 *a*	12 *b*	13 *a*	13 *b*	14 *a*	14 *b*	15 *a*	15 *b*	16 *a*
Late Red Clover	22	—	41	—	—	22	—	—	43	—	—	66	—	—	30	—	—
Broad Red Clover	—	—	—	29	23	—	—	59	—	—	61	—	—	—	—	—	—
White Clover	—	—	—	—	1	—	6	—	—	4	—	—	—	—	—	—	2
Alsike	—	—	—	—	—	—	—	—	—	—	—	—	20	—	—	—	6
Yellow Suckling	—	2	—	—	—	—	—	—	—	17	—	—	14	2	—	3	9
Black Medick	—	—	—	—	—	—	9	—	—	—	—	—	—	—	—	—	—
Sainfoin	—	—	—	—	—	—	—	—	—	—	—	—	—	—	44	—	—
Perennial Rye	1	2	51	60	1	—	77	17	48	50	24	17	52	82	—	14	2
Italian Rye	—	—	—	2	74	68	—	11	—	—	3	—	5	1	—	13	1
Cocksfoot	—	92	—	3	—	—	—	—	—	2	—	—	—	—	—	—	75
Timothy	75	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
R. S. Meadow Grass	—	—	5	—	1	1	3	11	4	7	7	3	5	4	21	1	3
Fescues	—	—	—	—	—	—	—	—	—	10	—	—	—	—	—	—	—
Tall Oat	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	66	—
Bent	2	4	2	2	—	—	—	1	—	7	4	8	—	6	5	1	1
Yorkshire Fog	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—
Soft Brome	—	—	—	—	—	1	—	—	—	—	1	—	—	—	—	—	1
Weeds	—	—	1	4	—	8	5	1	4	3	—	6	4	5	—	2	—

Table IV. 1926.

Percentage by weight of plants present in hay.

	8 *a*	8 *b*	9 *a*	9 *b*	10 *a*	10 *b*	11 *a*	11 *b*	12 *a*	12 *b*	13 *a*	13 *b*	14 *a*	14 *b*	15 *a*	15 *b*	16 *a*
Late Red Clover	13	—	41	—	—	19	—	—	45	—	—	29	—	—	34	—	—
Broad Red Clover	—	—	—	43	40	—	—	37	—	—	38	—	—	—	—	—	—
White Clover	—	2	1	—	—	—	17	—	—	7	—	—	4	4	1	—	3
Alsike	—	4	—	—	—	—	—	—	—	—	—	—	35	—	—	—	17
Yellow Suckling	—	2	—	—	—	—	17	8	—	3	—	—	19	30	2	24	49
Black Medick	2	—	2	14	—	—	—	—	—	27	—	—	—	—	—	—	—
Sainfoin	—	—	—	—	—	—	—	—	—	—	—	—	—	—	44	—	—
Perennial Rye	9	—	30	18	4	2	43	8	23	10	3	40	19	11	3	—	1
Italian Rye	—	—	—	—	26	12	—	14	—	—	—	—	5	—	1	—	—
Cocksfoot	—	60	—	—	—	—	—	—	—	2	—	—	—	—	—	4	24
Timothy	75	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
R. S. Meadow Grass	—	4	14	10	19	2	11	17	17	13	12	7	10	4	6	—	2
Fescues	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	4	—
Tall Oat	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	42	—
Golden Oat	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	10	—
Bent	—	22	1	3	3	—	3	1	5	29	40	21	5	29	7	3	—
Yorkshire Fog	—	—	—	1	—	18	2	1	4	—	1	1	—	6	—	1	—
Soft Brome	—	—	1	2	4	24	3	10	—	2	2	—	—	6	—	2	—
Weeds	1	6	10	9	4	23	5	4	6	7	4	2	3	10	2	7	2
Sweet Vernal	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—
Crested Dogstail	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	—

Table V. 1927.

Percentage by weight of plants present in hay.

	8 *a*	8 *b*	9 *a*	9 *b*	10 *a*	10 *b*	11 *a*	11 *b*	12 *a*	12 *b*	13 *a*	13 *b*	14 *a*	14 *b*	15 *a*	15 *b*	16 *a*
Late Red Clover	—	—	—	—	—	33	—	—	22	—	—	33	—	—	7	—	—
Broad Red Clover	—	—	—	—	14	—	—	45	—	—	1	—	—	—	—	—	—
White Clover	—	—	—	—	—	1	3	1	2	—	6	3	1	3	1	1	3
Yellow Suckling	—	—	—	—	—	—	—	—	—	—	—	—	10	8	1	4	10
Black Medick	—	—	—	—	13	—	—	1	—	—	10	2	—	—	—	12	—
Sainfoin	—	—	—	—	—	—	—	—	—	—	—	—	—	—	27	—	—
Perennial Rye	—	—	—	—	—	20	11	—	5	1	—	11	17	12	—	—	—
Cocksfoot	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	16
Timothy	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—
R. S. Meadow Grass	—	—	—	—	5	—	—	—	—	—	—	—	—	—	—	—	—
Fescues	—	—	—	—	—	—	—	—	—	—	—	—	—	2	—	15	—
Tall Oat	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	24	—
Golden Oat	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	3	—
Sweet Vernal	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	—
Bent	—	—	—	—	35	4	34	26	44	65	40	21	41	30	36	28	37
Yorkshire Fog	—	—	—	—	12	24	14	11	4	17	21	11	12	26	10	4	6
Soft Brome	—	—	—	—	4	11	6	8	12	14	17	9	11	15	18	6	19
Weeds	—	—	—	—	17	7	32	8	11	3	5	10	8	4	Trace	Trace	9

POLYSULPHIDE SULPHUR IN RELATION TO THE FUNGICIDAL EFFICIENCY OF CERTAIN SPRAY MATERIALS

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Indications of the fungicidal properties of sulphur in the polysulphide form were secured by Eyre and Salmon (2). Colourless solutions of ammonium hydrosulphide and of ammonium sulphide, on being allowed to stand exposed to the air, developed the yellow colour indicative of the formation of polysulphide and it was observed that this change was accompanied by an increased fungicidal efficiency of these solutions.

Hence arose the suggestion that the fungicidal powers not only of ammonium polysulphide solutions but also of lime sulphur, liver of sulphur and other polysulphide compounds may be determined by their content of polysulphide sulphur. This hypothesis has since been subjected to a critical examination upon the reactions of the hop powdery mildew (*Sphaerotheca Humili* (DC.) Burr.) to such solutions. Owing to the short period of the year during which the biological material is available, the work has already occupied some fifteen years. The results of these investigations have been published in a series of papers entitled "The Fungicidal Properties of certain Spray-fluids" (2, 3, 7, 8), which, however, partake of the nature of progress reports and which contain much matter irrelevant to this particular problem. The object of the present communication is to separate, survey and co-ordinate those results so far obtained which have a direct bearing upon the question in hand.

Soluble polysulphide compounds arise by virtue of the fact that solutions of ammonium and certain metallic sulphides are able to combine with free sulphur. By "polysulphide" sulphur is meant this additional sulphur—the sulphur present in sulphide form in excess of that required to form the normal sulphide. This latter form of sulphur has been called the "monosulphide" sulphur. The polysulphide sulphur may be detected and its amount estimated, since by the passage of carbon dioxide or by the addition of the salt of a metal forming an insoluble sulphide, it is precipitated as free sulphur. The sulphur present as monosulphide sulphur, on the other hand, is either removed as hydrogen

sulphide or as the metallic sulphide. The method employed throughout the greater part of the work described below to estimate the polysulphide sulphur depends upon its precipitation as free sulphur by the addition of zinc chloride (see (5)). For the estimation of the sulphur present as monosulphide sulphur, as thiosulphate, as sulphate and of the total sulphur present, the methods already described (6) were employed.

The fungus used in testing the fungicidal value of the various solutions was the hop powdery mildew. To avoid, as far as possible, variation on the part of the host plant with possible consequent effects on the vigour of the fungus, all the plants used were clone plants, *i.e.* plants raised vegetatively by cuttings from one individual hop plant which had proved very susceptible to the mildew. The plants were grown in pots in an unheated greenhouse under conditions "standardised" as far as possible. The stage of the fungus selected for spraying was the young "powdery" conidial stage produced on young leaves at the third to sixth node from the apex. For each trial ten or more leaves were used, each bearing several (4–20) powdery patches of the mildew whilst the same number of leaves at the same nodes bearing similar mildew patches were kept as controls. Where a comparative test of two solutions was required, the controls were used for the second spray. In this way a comparison was possible under similar biological and external conditions.

The spray fluids were applied by means of a hand atomiser throwing a fine mist-like spray. To ensure a thorough and complete wetting of the fungus a spreader, generally soft soap or gelatine, was added to the solution. In comparative work it was found necessary to employ the same concentration of the same spreader, for not only is the total amount of the spray retained on the leaf dependent upon the strength and nature of the spreader, but evidence was obtained that the spreader may have a direct influence upon the fungicidal efficiency of the spray.

The criterion employed to judge the extent of fungicidal action of the spray was the re-growth of fresh conidiophores from the sprayed mildew patches. The solution was considered fungicidal when no growth of conidiophores had occurred within ten days, a period sufficiently long to indicate the killing of the mycelium.

Experimental.

The hypothesis that the polysulphide sulphur content of the polysulphide group of fungicides gives a measure of their relative fungicidal efficiencies is confirmed by work which may be divided into the following sections.

1. Firstly, it was established that the total content of sulphur gives no index of fungicidal value as was shown by the work of Eyre, Salmon and Wormald (3), in which various solutions of ammonium polysulphide were used with 1 per cent. soft soap as the spreader. Their results are illustrated by the following examples:

Solution	Total sulphur gm. per 100 c.c.	Approximate fungicidal strength
II	9·1	Fungicidal at 1 : 30; nearly fungicidal at 1 : 50
III	29·1	Fungicidal at 1 : 30; very nearly fungicidal at 1 : 50
IV	24·2	Fungicidal at 1 : 100

Thus, solutions II and III have almost the same fungicidal efficiency, yet solution III contains three times as much total sulphur. Also, solution IV has less total sulphur than solution III, yet is about three times as effective as a fungicide.

2. Secondly, it was shown that the constituents of the polysulphide solution other than polysulphide sulphur are without appreciable fungicidal action. Our knowledge of the chemistry of the polysulphide solutions is still incomplete, but the known constituents include those sulphide compounds possessing sulphur in monosulphide and polysulphide form, the thiosulphate and (in the case of the alkali metals) the sulphate of the metal, whilst, according to some authorities, the sulphite and hydroxyhydrosulphide of the metal are suspected constituents. Finally, owing to the hydrolysis of the polysulphide compounds, the presence of traces of hydrogen sulphide and free hydroxyl ions has been demonstrated by Goodwin and Martin (6). It must be pointed out that similar products of hydrolysis will, however, appear in solutions of the hydroxyhydrosulphide. Horton and Salmon (8) examined the fungicidal properties of the following possible constituents of lime sulphur solutions at strengths well above those present in a fungicidal solution of lime sulphur:

Calcium sulphate (saturated solution)	Non-fungicidal
Calcium sulphite (5 per cent. suspension)	,,
Calcium thiosulphate (0·5 gm. thiosulphate sulphur per 100 c.c.	,,
Calcium hydroxyhydrosulphide (0·85 gm. monosulphide sulphur per 100 c.c.)	,,

In the above experiments, a suspension of calcium caseinate containing 1 per cent. casein and 0·4 per cent. calcium oxide was employed as the spreader.

Further, Eyre and Salmon (2) not only showed a solution of 1 per cent. sodium thiosulphate (approximately 0·26 gm. thiosulphate sulphur per

100 c.c.) and 1 per cent. soft soap to be non-fungicidal, but proved Foreman's contention (4) that free alkali is the potent fungicidal agent of liver of sulphur to be untenable by finding a solution containing 0·5 per cent. caustic soda plus 1 per cent. soft soap to be non-fungicidal. The fungicidal properties of hydrogen sulphide against *S. Humuli* have not been closely examined, but Eyre and Salmon showed that a solution containing 0·056 per cent. sulphur as hydrogen sulphide plus 1 per cent. soft soap was non-fungicidal.

3. Thirdly, it has been shown that the presence of varying amounts of the possible constituents (in particular, sulphur as thiosulphate and in the form of monosulphide sulphur) has no influence upon the fungicidal effectiveness of the polysulphide sulphur.

That the sulphur in monosulphide form has no effect upon the direct fungicidal efficiency may be deduced from the results secured by Eyre, Salmon and Wormald already quoted in section 1. Unfortunately, methods for the direct estimation of polysulphide sulphur were, at that time, unavailable. It may be assumed, however, that the amount of thiosulphate sulphur initially present in the ammonium polysulphide solutions they employed was negligible[1] for, by the method of preparation, oxygen was excluded by the ammonia and hydrogen sulphide present. The figures quoted by Eyre, Salmon and Wormald for "sulphide" sulphur are, from their method of estimation, those which in later work have been called monosulphide sulphur; the figures for polysulphide sulphur may then be obtained by difference. Despite the possible inaccuracy of the polysulphide figure, the agreement shown in the following example is sufficient to permit the conclusion that the relatively large amount of monosulphide sulphur in solution III has not affected the fungicidal activity of its polysulphide sulphur:

Solution	Gm. per 100 c.c. "Sulphide" sulphur	Gm. per 100 c.c. Polysulphide sulphur	Approximate fungicidal strength
III	24·6	4·5	Fungicidal at 1 : 30
IV	10·6	13·6	Fungicidal at 1 : 100

In trials, more strictly comparable in that the solutions were contrasted by spraying upon opposite leaves at the same node, Eyre, Salmon and Wormald arrived at similar results of which the following may serve as examples:

[1] Solutions prepared by the method used for solutions III and IV below were found to contain 0·12 and 0·17 gm. thiosulphate sulphur per 100 c.c. respectively, when analysed within 24 hours of preparation.

Ammonium polysulphide solutions plus 1 per cent. soft soap.

Solution	Polysulphide sulphur gm. per 100 c.c.	Ratio monosulphide sulphur/polysulphide sulphur	Effect on fungus
VIII	0·077	0·56/1·16	Fungicidal
XI	0·078	5·23/15·7	,,
VIII	0·053	0·56/1·16	Nearly all the patches killed; a very few patches with a few, usually scattered, conidiophores
XI	0·052	5·23/15·7	Nearly all the patches killed; a few patches with clustered conidiophores at the edges
V	0·077	2·6/1·23	Fungicidal on 8 leaves, very nearly fungicidal on 2 leaves, not quite fungicidal on 1 leaf
VII	0·077	3·8/3·4	Fungicidal on 7 leaves, very nearly fungicidal on 2 leaves, not quite fungicidal on 1 leaf (1 leaf died)

An important corollary follows from this conclusion, namely, that the fungicidal efficiency of the polysulphide sulphur is the same whether the lower polysulphides or the higher predominate in the solution. It is the ratio of monosulphide sulphur to polysulphide sulphur which determines the status of the polysulphide compounds present. Thus, in solution VIII above, the analysis shows that the ammonium polysulphide compound present approximates closely to the formula $(NH_4)_2S.S_2$, whereas the greater part of the polysulphide sulphur present in solution XI would be in the form represented by the formula $(NH_4)_2S.S_3$.

That the amount of thiosulphate sulphur initially present in the polysulphide solution does not adversely affect the activity of the polysulphide sulphur may be illustrated by the following examples obtained in experiments in 1929[1]. In each case recorded below, the effects of external conditions have been eliminated by spraying opposite leaves at the same node with various calcium polysulphide solutions, using 0·5 per cent. gelatine as the spreader:

Ref. no.	Polysulphide sulphur gm. per 100 c.c.	Ratio thiosulphate sulphur/polysulphide sulphur	Effect on mildew
52/29	0·108	33·0/100	Fungicidal
51/29	0·108	3·2/100	,,
61/29	0·096	27·0/100	Fungicidal
62/29	0·100	2·3/100	,,

4. Finally, the point was established that the fungicidal efficiency of the polysulphide sulphur was independent of the base present. It was considered possible by Eyre, Salmon and Wormald (3) that, since different ammonium polysulphide solutions containing equal amounts of poly-

[1] Fuller details of the 1928 and 1929 experiments with polysulphide sprays will be published in "The Fungicidal Properties of certain Spray-Fluids, Part VII."

sulphide sulphur behaved similarly as fungicides, ammonium polysulphide solutions and lime sulphur solutions having the same polysulphide sulphur content might have the same fungicidal action. Employing 1 per cent. saponin as the spreader they were able to show, despite possible inaccuracies in the polysulphide figure, an agreement sufficiently close to warrant further trials:

Ammonium polysulphide (solution XI) and lime sulphur (solution XII).

Solution	Polysulphide sulphur gm. per 100 c.c.	Effect of solution on mildew
XI	0·078	All patches killed on 4 leaves; on 6 leaves some patches killed, but many only more or less checked
XII	0·078	
XI	0·113	Almost fungicidal on 4 leaves; patches severely checked on 4 leaves, slightly checked on 2 leaves
XII	0·113	Patches severely checked on 5 leaves, slightly checked on 5 leaves

In 1923, we (7) were able to compare potassium polysulphide solutions (prepared from a commercial liver of sulphur) and sodium polysulphide solutions (prepared from the commercial product "Sulfluid") with the following results, 1 per cent. soft soap being the spreader used:

Liver of sulphur (A) and "Sulfluid" (B).

Ref. no.	Polysulphide sulphur gm. per 100 c.c.	Effect on mildew
A 22	0·03	By the tenth day, all patches on 1 leaf sterile; on 6 leaves some patches showed fresh scattered conidiophores while other patches were sterile
B 22	0·03	By the tenth day, fresh scattered conidiophores arising from some patches on 5 of the leaves while the other patches and all those on the remaining 2 leaves were dead
A 13	0·121	Fungicidal
B 12	0·12	"

The composition of the two commercial products used was:

	Liver of sulphur		"Sulfluid"	
	%		%	
Polysulphide sulphur	27·56	100·0	7·05	100·0
Monosulphide sulphur	9·28	33·7	1·57	22·3
Thiosulphate sulphur	8·56	31·1	0·41	5·8
Sulphate sulphur	2·01	7·3	0·73	10·4

Despite the differences in the amounts of sulphur compounds associated with equal polysulphide sulphur, the two sprays behaved similarly as fungicides.

In continuation of this work, two solutions of sodium and potassium polysulphides were prepared in the laboratory by the passage of hydrogen sulphide into solutions of the hydroxides, the resultant mixture being boiled with excess of sulphur. Spraying trials, using 1 per cent. soft soap as the spreader, gave the following results:

Potassium polysulphide (*A*) *and sodium polysulphide* (*B*) *solutions.*

Ref. no.	Polysulphide sulphur gm. per 100 c.c.	Effect on mildew
A 21/28	0·099	Fungicidal
B 20/28	0·097	,,
A 29/28	0·052	Fungicidal
B 30/28	0·052	,,
A 38/28	0·05	Fungicidal
B 37/28	0·05	,,

The ratio of total sulphur to polysulphide sulphur in these two solutions was in *A*, 6·24 to 4·11, and in *B*, 6·53 to 4·16, the two solutions therefore being somewhat similar in composition. It is obvious that the substitution of sodium hydroxide for potassium hydroxide has had no influence upon the fungicidal properties of the polysulphide sulphur in the solutions.

In the same year (1928), methods of analysis of barium polysulphide solutions were evolved and it became possible to contrast solutions of barium polysulphide with the other polysulphide sprays. A trial, employing 0·5 per cent. gelatine as the spreader, gave the following result:

Ref. no.	Polysulphide sulphur gm. per 100 c.c.	Effect on mildew
34/28	0·102	Practically fungicidal; very rarely more or less clustered conidiophores from the youngest patches
33/28	0·104	Practically fungicidal; very rarely more or less clustered conidiophores from the youngest patches

Of the above, 33/28 was prepared from the barium polysulphide and it is evident that the polysulphide sulphur is as effective as that in the solution of sodium polysulphides (34/28).

5. To confirm the above deductions, a comprehensive comparison of various polysulphide sprays was undertaken. The stock materials used included two commercial products, one, a lime sulphur, the other,

"Amberene," a solution of sodium polysulphides. The remaining six samples were prepared in the laboratory; samples 13, 14, 15 and 16 were prepared by passing hydrogen sulphide into solutions of the hydroxide of the metal and boiling the resultant mixture with sulphur. After cooling, the solutions were filtered and stored in air-tight bottles. Two calcium polysulphide solutions (samples 17 and 18) were prepared by boiling mixtures of hydrated lime, sulphur and water, the cooled solutions being filtered before use. Attempts to prepare a solution of calcium polysulphides containing higher proportions of monosulphide sulphur failed, probably owing to the precipitation of Herschell's crystals (see (1)).

Analysis of the various stock solutions gave the figures quoted in Table I, with which is included the proportions of other sulphur compounds present, calculated upon the basis of 100 parts of polysulphide sulphur:

Table I.

Sample no.	11 Lime sulphur		12 Amberene		13 Sodium polysulphide		14 Potassium polysulphide	
	%		%		%		%	
Polysulphide sulphur	19·20	100·0	11·82	100·0	5·26	100·0	3·09	100·0
Monosulphide sulphur	5·04	26·2	3·74	31·6	1·30	24·7	0·76	24·6
Thiosulphate sulphur	0·62	3·2	5·18	43·8	0·32	6·1	0·09	2·9
Sulphate sulphur	0·00	0·0	0·00	0·0	0·00	0·0	0·01	0·3
Total sulphur	—	129·4	—	175·4	—	130·8	—	127·8

Sample no.	15 Barium polysulphide		16 Calcium polysulphides		17		18	
	%		%		%		%	
Polysulphide sulphur	3·36	100·0	6·53	100·0	8·76	100·0	5·67	100·0
Monosulphide sulphur	0·74	22·0	1·46	22·4	2·00	22·8	1·45	25·6
Thiosulphate sulphur	0·05	1·5	0·15	2·3	2·37	27·0	1·87	33·0
Sulphate sulphur	—	—	0·00	0·0	0·00	0·0	0·00	0·0
Total sulphur	—	123·5	—	124·7	—	149·8	—	158·6

For the preparation of the sprays, the stock solutions were diluted to the requisite amount and an equal volume of 1 per cent. gelatine added. The polysulphide sulphur figure quoted below was determined by the analysis of the diluted spray.

In the first six experiments above (Ref. nos. 7–12/29) the strength of solution aimed at was 0·10 gm. polysulphide sulphur per 100 c.c. For the last eight experiments the strength of solution was increased to

Ref. no.	Sample no.	Polysulphide sulphur gm. per 100 c.c.	Effect on mildew
{ 7/29	13	0·096	Non-fungicidal to not quite fungicidal
{ 8/29	11	0·100	,, ,, ,, ,,
{11/29	15	0·106	,, ,, ,, ,,
{12/29	16	0·091	,, ,, ,, ,,
{ 9/29	17	0·102	Apparently not quite fungicidal
{10/29	14	0·099	,, ,, ,,
{17/29	13	0·107	Fungicidal
{18/29	11	0·108	,,
{24/29	15	0·117	,,
{25/29	16	0·109	Fungicidal or just below fungicidal strength (like 26/29)
{26/29	14	0·109	Fungicidal or just below fungicidal strength
{27/29	17	0·112	,, ,, ,, ,,
{30/29	12	0·114	Fungicidal (1 leaf with scattered conidiophores)
{31/29	18	0·109	Fungicidal or occasionally not quite fungicidal

0·11 gm. per 100 c.c. Theoretically, only the bracketed trials, carried out on opposite leaves at the same node, need yield similar results for similar polysulphide sulphur content. The agreement between individual members of each group is so consistent, however, that the use in each pair of trials of one particular solution as a standard was considered unnecessary. It is probable that, in the short period intervening between successive sprayings (two days between Exps. 7–12/29; nine days between Exps. 17–31/29), factors such as temperature and age of host plant have had little effect upon the response of the fungus. A direct comparison of the individual members within each group therefore becomes possible.

Hence the results confirm the main contention, that the content of polysulphide sulphur supplies an index to fungicidal power. As will be seen from the table of analyses, in spite of differences in:

(1) the total sulphur content (the ratio varies from 123·5 in the case of the barium polysulphide solution to 175·4 for Amberene);

(2) the monosulphide sulphur content (22·0 in sample 15 to 31·6 in sample 12);

(3) the thiosulphate sulphur content (1·5 in sample 15 to 43·8 in sample 12);

(4) the various bases employed;

the behaviour of the solution as a fungicide towards *S. Humuli* is in accordance with its content of polysulphide sulphur.

Summary.

The results of a prolonged investigation of the fungicidal properties of solutions containing sulphur in polysulphide form upon the hop powdery mildew (*S. Humuli*) have been classified and summarised. During the course of this work the fungicidal efficiency of various lime sulphurs, liver of sulphurs, and solutions containing sodium, potassium, ammonium, calcium and barium polysulphides have been compared and it has been shown that, in all cases, the effect upon the fungus, when growing under similar biological and external conditions and provided that the sprays are applied under standardised conditions, is determined solely by the content of polysulphide sulphur of the spray fluid. The estimation of the amount of polysulphide sulphur present provides, therefore, a measure of the efficiency of these materials against *S. Humuli*.

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ON THE CAUSES OF SWARMING IN THE HONEY BEE (*APIS MELLIFERA* L.): AN EXAMINATION OF THE BROOD FOOD THEORY

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(With 3 Text-figures.)

Introduction.

Up till comparatively recent times, bees were kept in hives of various types in which the combs were fixed, beekeepers had little knowledge of what took place in the hive and even less power of control. Loss of colonies was consequently frequent. In England the straw skep was the hive in common use, and the custom was to suffocate a proportion of the stocks of bees in autumn over burning sulphur for the sake of their honey. Natural swarms were necessary to replace those stocks destroyed by the beekeeper as well as losses occasioned by other causes, since the formation of artificial swarms and nucleus colonies are operations which were not general before the introduction of the movable frame hive. Beekeepers of the present day prefer to make artificial increase, to suit their own requirements. Natural swarming involves loss of time to the beekeeper at a busy season of the year, loss of working strength and interruption of breeding in the colony, resulting in a reduced crop of honey, and possible loss of the swarm. In modern apiaries, therefore, every effort is made to control swarming or to prevent it altogether.

It is not intended in this review to consider the significance of swarming in the evolution of the social Hymenoptera, nor the possibility of producing strains of bees less prone to swarm, by selection and breeding; but to examine the brood food theory of Gerstung in the light of modern knowledge of the biology of the bee.

The brood food.

Honey, a carbohydrate food, is used by the adult bees as the source of heat and energy in the work of the hive, particularly in the production of heat for the maintenance of temperature in the winter cluster, and for the incubation of brood during the early part of the year.

The source of nitrogen (protein) in the hive is pollen, the consumption of which is typical of the growth phase of colony activity. Pollen is consumed by young worker bees; it is given together with honey to the older larvae of the worker and drone castes, and it is used indirectly in the preparation of the special brood food which we are now about to discuss.

In this paper the term "brood food" is used in the special sense of the elaborated food which is given to the younger larvae of all castes, as opposed to the pollen-honey mixture, which worker and drone larvae receive from the third (13) or fourth day onwards[1].

The fertilised (female) egg is potentially a queen, and will become one if fed throughout its larval existence on the richer food: workers are imperfect females produced under a system of nutritional castration by being weaned from the prepared food at an early age. The age at which the diet is changed was given by von Planta as the fourth day. Nelson and Sturtevant (13) have shown more recently that the change takes place on the third day.

Composition of the brood food.

The analyses of von Planta (16, 17) make it appear that the food of young larvae differs in composition from that given to those of drones of the same age, and also from the "royal jelly" received by the queen larvae of all ages. Koehler (8) gives figures for fat and sugar, which, differing from those of v. Planta, appear to indicate that the food of the young worker and drone larvae are identical. Aeppler (1) has also made analyses of the royal jelly[2].

Elser (30) has also studied this problem, but his results (31) appeared too late for inclusion in this paper.

Table I.

Composition of brood food.

(Expressed as percentage of dry weight.)

	Queen (average)		Worker (under 4 days)		Drone (under 4 days)	
	v. Planta	Aeppler*	v. Planta	Koehler	v. Planta	Koehler
Protein	45·14	40·36	53·38	—	55·91	—
Fat	13·55	20·05†	8·38	23·3	11·90	24·23
Sugar	20·39	18·52	18·09	15·7	9·57	14·9

* See note 2 below. † Total ether extract.

[1] The term "chyle food," which is often used in England, has been carefully avoided in this paper, for reasons given later.

[2] Aeppler's figures were given as follows: "Larval food in air dried condition.... Moisture after drying at 100° C. 24·15 per cent.; total protein 30·62 per cent.; total sugars 14·05 per cent.; total ether extract 15·22 per cent." The figures given in the table are calculated on the dry matter obtained at 100° C. as stated by him.

Origin of the brood food.

Two opposing views have been held as to the origin of the brood food.

(*a*) *The glandular secretion theory.* According to Schiemenz (21) it is a secretion of the lateral pharyngeal glands of the head, which are found in their greatest development in the social bees, and which are physiologically active in those bees that are engaged in nursing the young larvae. This view is shared by Cheshire (3) and by most modern writers (8, 29).

(*b*) *The regurgitation theory.* This was put forward by Schönfeld (22) and has been championed by Cowan (5), Cook (4) and others. This theory maintains that the brood food consists of a pre-digested food regurgitated from the ventriculus or so-called "chyle stomach" of the worker bee. In order to bring this about the honey stomach was said to be capable of being "short circuited" by pushing the stomach-mouth forward against the end of the oesophagus. Schönfeld claimed to have brought this about experimentally, but other observers say that such an action is impossible (12, 24, 28). The supporters of the regurgitation theory base their claim on the fact that insoluble matter such as lamp black, supplied experimentally to bees, has been recovered in the food given to the larva, whereas no solid matter would pass through the gland (4). However, this might easily be brought about through mechanical contamination of the mouth parts. The name "chyle food," current in this country, is based on this view[1]. Arnhart[2] seems to adopt an intermediate position by stating that brood food is a combination of regurgitated contents of the stomach with the glandular secretion which changes its reaction from alkaline to acid. The arguments on both sides are summarised by Snodgrass in his bulletin on the *Anatomy of the Honeybee* (24). In his more recent book (25) this is omitted, for he says: "It is now generally conceded that the pharyngeal glands are the organs which form the brood food or royal jelly."

Langer (9) in 1912 proved by biochemical methods that the proteins of the brood food are identical with those of the head gland. This is a further strong argument in favour of the secretion theory.

[1] The word *chyle*, used in vertebrate physiology to denote the contents of the lacteal vessels, corresponds to the German *Futtersaft*. The word *Futterbrei*, used by von Planta, should properly be translated *chyme*, and is more appropriate in connection with the regurgitation theory.

[2] Quoted by Snodgrass (24).

The brood food theory of swarming.

About the year 1891 Gerstung, in Germany, propounded his brood food theory, which he subsequently elaborated in considerable detail, emphasising the view that the bee colony is to be considered as a unit, and not as a fortuitous collection of individual bees. He writes of "Der Bien" (7) as an organism of which the constituent bees are members (organs) carrying out various functions. On this view the queen is the generative organ of the colony, and swarming is an act of reproduction.

Continuing this analogy the brood food may be compared to an endocrine secretion, a surplus of which creates a special condition within the hive leading to preparations for swarming. It is considered that when nurse bees, having the brood food glands in a state of activity, exist in excess of the requirements of the brood in the hive, there is a tendency to build queen cells. Crudely stated, it may be said that the surplus is given to certain favoured larvae in order to get rid of it. These larvae develop into queens, and when the cells are sealed the colony is liable to give off a swarm (2).

Division of labour in the bee colony.

The work of Rösch (19) on the biology of the bee has a direct bearing on the brood food theory. He makes the following statement: "Every worker bee is able to undertake all the tasks which present themselves; following—with advancing age—a definite sequence which is the same for each individual." According to him the life of a worker bee may be divided into three periods, which we may call (1) nursery work, (2) house work, and (3) field work. Specialisation such as we find in ants does not appear to exist in the honey bee.

The first duty of a newly hatched bee is to prepare the cells for the next batch of eggs and to do her share of keeping up the temperature of the nest by "brooding over" the developing larvae. From about the third to the sixth day she feeds the older larvae with pollen and honey taken from the store cells. The food glands are developing during this time in consequence of the rich food taken. From the sixth to the tenth or fifteenth day, the food glands being fully developed, she attends to the feeding of the very young larvae which are receiving the prepared brood food. "The end of the brood nursing period is not determined by age. When nurses are scarce it can be prolonged, but in normal stocks it does not continue beyond the 13–15th day. By this time the gland has atrophied again" (19).

The second period begins with the first flight from the hive, the so-

called orientation flight. During this period the bee receives nectar from the foragers, ripens and stores it; presses down pollen loads into the cells where they have been deposited, and acts as house cleaner. It is bees of this age that secrete wax when there is building to be done (20). Towards the close of the second period the bee may become a guard at the entrance.

The bee then becomes a forager (third period) and gathers water, pollen, nectar and propolis; she continues at this work until overtaken by accident or old age.

The following table compiled in part from data given by Rösch gives the normal succession of duties.

Table II.

Life of a worker bee.

Stage	Duration (days)	Age (days)
Development:		
Egg	3	1–3
Larva (unsealed) (weaned on 3rd day)	5	4–8
Sealed brood	12	9–21
Adult bee:		
1st period: Nurse bees		
Incubating brood preparing brood cells	2–3	1–3
Feeding older larvae with honey and pollen ...	3	3–6
Feeding young larvae with brood food	4–9	6–10 (up to 15)
2nd period: House bees		
First play flight ... ... Storekeeper (receiving, ripening and storing nectar) House cleaner ... ... Wax secretion ... ... Guard (at the hive entrance)	10	10–20
3rd period: Field bees		
Forager (water, pollen, nectar, propolis)	20–30	20–40 up to 50

THE BROOD REARING CYCLE.

The seasonal activity of the queen has been studied by Nolan (14) and is somewhat as follows: After the winter rest breeding starts on quite a small scale and increases until a maximum is reached early in the season, after which it falls off rapidly, though there may be a secondary peak in autumn (Fig. 1).

RELATION OF THE EGG LAYING CURVE TO THE ONSET OF SWARMING.

Assuming that there are no casualties among eggs or larvae, the curve of emerging bees will be parallel with that of oviposition, 21 days later. These bees, according to Rösch, will be at the height of their nursing

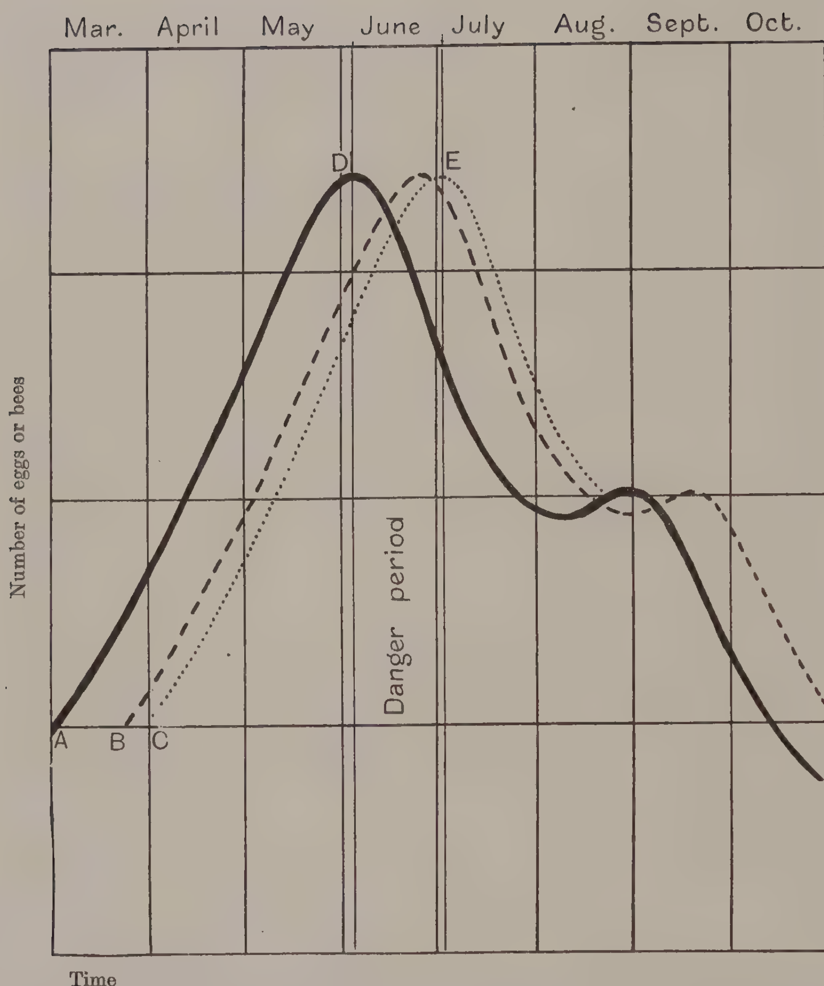

Fig. 1. Hypothetical brood curve of a colony of bees (adapted from those of Nolan[14]). A. Egg laying curve of queen rising to a maximum at D. B. Curve of emergence of adult bees; on the assumption that there have been no casualties among the brood. C. Shows the number of bees attaining the critical age, at which the brood food glands reach their full development. It is evident that the number of nurse bees (C) at any given period would be from four to nine times that represented on the curve, seeing that the duration of this period is from four to nine days (see Table II).

activities—*i.e.* at the critical age—in a further 6 to 10 days, that is to say, 25 to 30 days after they were themselves receiving the brood food.

In the early part of the year, as the number of nurse bees and the abundance of brood food in the hive increases, the queen herself receives a share of the prepared nitrogenous food and is stimulated to increase her output of eggs. Thus egg laying increases at a progressively steeper rate until a maximum is reached, which may be determined by the fertility of the queen, by the capacity of the brood chamber of the hive, or by some factor which we do not yet understand.

The egg laying curve then begins to fall off, and, parallel with it and four days later, the curve of young larvae which are receiving the brood food. The curve of emerging bees, and that of bees attaining the nursing age, continues to rise, resulting in an increasing surplus of brood food for which there is no outlet. This leads to the construction of queen cells, which will contain larvae capable of absorbing relatively large quantities of brood food.

VARIATIONS IN CYCLE OF DUTIES.

Rösch speaks of the division of labour as being flexible, without a hard and fast time schedule. Moreover, it is recognised that autumn-born bees are capable of raising brood and even queens in the following spring. When there exists in the hive a need for bees to carry out certain specified duties, it would seem that other younger bees are drafted on to the more mature tasks. Thus, while there is work to be done which properly belongs to the second period, nurse bees will continually be promoted to storekeeper or wax worker, while a need for foragers (third period) will produce vacancies in the ranks of the second period bees, which will, in their turn, have to be filled. Beekeepers know the kind of hive which is "too busy to swarm," and they are also only too familiar with the effect of enforced idleness, due to bad weather supervening when strong colonies have been built up for the harvest. It is commonly stated in books on beekeeping that a condition which leads to swarming is "congestion of bees in the brood nest." Now the bees which chiefly frequent the brood nest are those whose duties take them there, in other words, the brood nurses. This statement is therefore tantamount to saying that swarming is due to a superabundance of nurse bees, and therefore of brood food.

VALIDITY OF THE BROOD FOOD THEORY.

Even if the regurgitation hypothesis of Schönfeld were eventually proved to be correct, as Gerstung himself believed, this would not invalidate the brood food theory. Rösch and Soudek (26) have both shown

that the pharyngeal gland reaches its greatest development at the time when bees are engaged in brood feeding. It might be argued that this gland is merely concerned with the digestion of protein, at a time when bees are consuming large quantities of pollen for the elaboration of brood food.

"Control bees."

The idea has been put forward recently in America (10, 11) that the policy of the hive is determined by bees of between 14 and 21 days old. Whereas young bees are devoted to nursery duties, and old bees are fully occupied with foraging, these bees of middle age can turn to any task. These latter are the entrance guards, the scouts and the bees which compose the swarm. An abundance of such bees is the factor which decides the issue of a swarm. They have been called "control bees." It will be seen that these are the bees of Rösch's second period. It will also be obvious that the surplus of nurse bees of "critical age," which led to the starting of queen cells, by the time the cells are sealed and the colony is ready to swarm, will have become a surplus of bees of the second period, *i.e.* "control bees." The fact that these bees are also of the age to secrete wax, and before coming out have gorged themselves with honey, accounts for the facility with which comb is built by a swarm.

Swarm control measures.

The measures of swarm prevention, which are advocated on the recommendation of practical experience (6, 18, 27), mostly depend for their efficacy on manipulations which have the effect of removing some of the nurse bees from the brood nest. Conversely those conditions which lead to a surplus of these bees in the hive bring on the swarming fever.

(a) *Capacity of the brood nest.*

Restriction of the brood area, by causing a sudden drop in the oviposition curve when the limit is reached, aggravates the danger period following the peak of egg laying, and leads to swarming (Fig. 2). This may be brought about by restricted hive accommodation, through the presence of unsuitable comb composed of cells unfit to contain brood, or by a rim of sealed honey which prevents expansion.

(b) *"Building up" period.*

"The tendency to swarm is greatest in those localities in which the bees increase brood rearing most rapidly in spring...during those years when the bees build up in the shortest interval in spring...in those

colonies which reach their peak of brood rearing most rapidly" (6). It will be seen on reference to Fig. 2 that the more rapid the rise just before reaching the peak, the more acute will be the surplus of nurse bees immediately following it. One of the advantages of spring protection (15) is

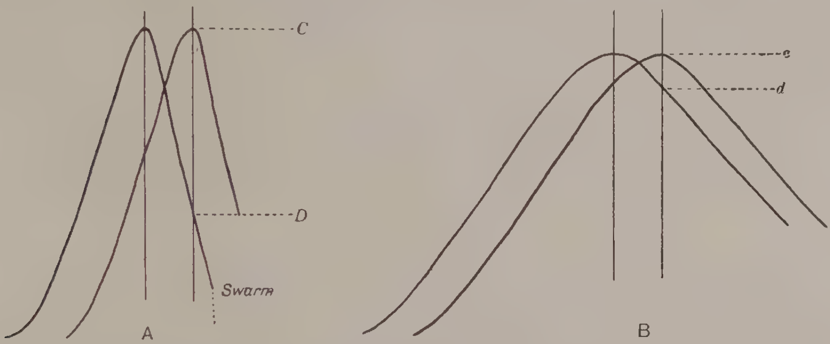

Fig. 2. Effect of shape of brood curve at its maximum on the tendency to swarm. A. A rapid fall from the maximum of egg production, giving rise to a strong tendency to swarm as indicated by the surplus of nurses over egg production *CD*. B. Brood curve falling off gradually from maximum, producing a less acute surplus of nurse bees and consequently a less marked tendency to swarm, as indicated by the vertical distance *cd*.

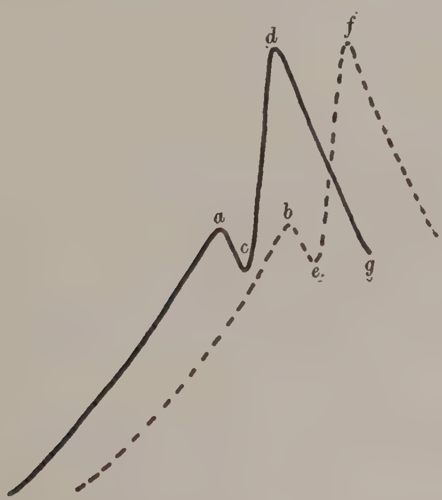

Fig. 3. Effect of check in spring brood rearing (at *a*), producing a temporary surplus of nurse bees at *b* which stimulates the queen producing a sharper rise in the brood curve *cd*. The falling off of nurse bees *be*, if it coincides with the peak of egg laying *d*, would hasten the decline of the egg curve, and produce a critical condition represented by the vertical distance *fg*. (Black line = brood: dotted line = nurse bees.)

that brood rearing increases at a steady rate without fear of checks due to spring frosts. Contrast with this the hurried brood rearing of an unpacked hive, starting late and increasing rapidly to a peak. In this connection it has been claimed that one of the benefits of spring protection lies in the absence of checks in the upward trend of the brood curve, which are said to be reflected afterwards in a tendency to swarm. A check in the upward curve of egg laying before the maximum has been reached will be followed in four or five days' time by a surplus of brood food, owing to the absence of larvae to feed. This surplus being supplied to the queen would lead to a spurt in the egg laying, followed in about 30 days by a corresponding excess of nurse bees (see Table II and Fig 3).

(c) *Effect of honey flow.*

The advent of a honey flow, beginning just before colonies have reached swarming point (the critical stage) may cause colonies to divert their energies to foraging, to the neglect of swarming. This simply means that bees are promoted from house bee to forager, and the house bees are in their turn recruited from the ranks of the nurse bees, while there is work for plenty of house bees to do in the supers, away from the brood nest, storing and ripening the honey and providing wax for cells and cappings to contain it. On the other hand, a spell of bad weather, confining foragers to the hive, delays promotion and allows nurse bees to accumulate.

(d) *Wax secretion.*

Conditions which impel the bees to build comb provide work belonging to the second period to which nurse bees can be promoted; at the same time removing them from the brood nest. The mere provision of space or sheets of foundation is not always enough to bring this about. Simmins (23) says that bees will not swarm until there is comb reaching to the entrance of the hive. He therefore advocates providing for comb building below the brood nest as a measure of swarm prevention.

(e) *Effect of age of queen.*

Colonies headed by young queens are apparently less liable to swarm than those that have old ones. The influence of the young queen began in the previous season, when she continued brood rearing later into the autumn so that the stock went into winter quarters with younger bees. In the new year the young queen starts earlier and increases the brood nest at a steady rate, being less liable to be put off by conditions which would cause a check in the oviposition of an older queen.

(f) *Removing bees and brood.*

It is not an uncommon practice to make nucleus colonies or artificial swarms from stocks which might be expected to swarm. By so doing sealed brood (which will require no more feeding, but will shortly give rise to nurse bees) and bees from the brood nest (nurse bees) are removed from the parent colony; the result being the same as the check in the emergence of young bees, which results after the departure of a natural swarm. Thus the colony is immediately put into the condition of a colony that has swarmed: there is no surplus of bees of critical age, and the impulse to raise queen cells no longer exists.

(g) *Separation of queen and brood.*

Most systems of swarm prevention are based on the separation of queen and brood. To this class belong the Demaree system and its modifications, wherein the queen is placed below a queen excluder and the brood above. This has the effect of removing the restriction of space on the activities of the queen. Part of the emerging bees is kept above the excluder to care for the brood, and there is less congestion of nurse bees in the part occupied by the queen. Queen cells are liable to be built above the excluder, but the apiarist expects these and takes care to destroy them.

(h) *Influence of drones.*

The rearing of excessive numbers of drones has been condemned as an expensive luxury, but it might be well to bear the following aspect of the matter in mind. The presence of drones in a hive undoubtedly has some effect on swarming, probably in ways not directly connected with the brood food theory. The production of drone brood and ministering to the food requirements of the drones themselves provide an outlet for brood food, which does not lead to the production of yet more nurse bees (7).

(i) *Influence of temperature and ventilation.*

Excessive heat and lack of ventilation are conditions leading to swarming. It may be that these causes operate directly, or it may be that they stimulate physiological activity of the food glands, just as warmth is necessary for wax secretion. In many cases they are symptoms of the congestion of bees in the hive, which is itself the cause of the desire to swarm.

SUMMARY.

1. The influence of nitrogenous food in the hive is discussed in its bearing on the question of swarming.

2. Theories of the origin of the brood food are examined.

3. The division of labour among the bees of various ages is considered in its relation to the brood rearing cycle.

4. A critical surplus of nurse bees is found to be associated with the formation of queen cells in preparation for swarming.

5. Recognised swarm control measures are reviewed in the light of the brood food theory.

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THE BIOLOGY OF THYSANOPTERA, WITH REFERENCE TO THE COTTON PLANT

V. THE RELATION BETWEEN THE DEGREE OF INFESTATION AND THE TYPE OF SOIL

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(*University of Manchester.*)

(With 7 Text-figures.)

The question whether the type of soil in which plants are growing has any effect on the infestation of that plant by thrips is not one which has received much attention from workers on the Thysanoptera, though there are suggestions in a few papers that this question is worth consideration. Moulton (4), in his paper on the orange thrips (*Euthrips citri*), states: "the thrips is not so prevalent on trees planted in sedimentary or loam soils as where the soil is of a clayey or adobe texture. This fact may be explained as follows: this thrips, like most others of its group, presumably spends the last of its larval, its pupal, and its early adult life in the soil under the trees, and would naturally then be more or less affected by the texture of the soil and by cultivation. Orange groves are usually irrigated several times during the summer and are cultivated throughout the year. Sedimentary soils break to pieces readily when thus moistened and cultivated, and thrips in this ground would probably be broken from their small cells...and many of them would be killed by the cultivator and by the grinding together of the soil particles during cultivation. On the other hand, in clay lands the particles of soil pack closely together and form clods, and during cultivation any number of thrips within these clods might be repeatedly turned over and over without injury." In a later paper on the orange thrips (Jones and Horton (2)), however, it is stated that attempts to destroy the pupae by cultivation of the ground were unsuccessful.

In his paper on the pea thrips (*Kakothrips robustus*)—a soil pupating species—Williams (9) states that "the pea thrips is most prevalent on light soils. In the majority of the cases reported to me the soil has been light or gravelly, and a correspondent at Shoreham, Kent, states that the

damage is always worse in the village where the soil is light than at Highfield near by where the soil is heavier."

Cameron and Treherne (1), writing on the pear thrips, state that the insects do not penetrate so deeply into clay soil as into other lighter soils, but at the same time they say that cultivation of the soil is of doubtful value, for not only may it fail to kill the pupae but it may encourage the emergence of the adult insects.

Urich (7) finds that the attacks of cacao thrips are worse on clay soils, but in this case the thrips (*Selenothrips rubrocinctus*) does not leave the plant but pupates on the leaf, so the soil conditions would have little effect on the insect except in so far as they affected the general health of the plant.

The results of several years' experiments on *Thrips tabaci* in reference to the cotton plant (3) suggested that the soil texture is of great importance in the case of this thrips at least, so it was decided to carry out experiments to test the effect of different types of soil on *T. tabaci*. Experiments on cotton grown in a medium clay loam soil had shown that the thrips attack is definitely worse if this soil is tilled and not allowed to form surface cakes, so it was assumed from this that plants grown in a light soil would be more infested by thrips than similar plants grown in a heavy clay soil which easily formed surface crusts; therefore two very different types of soil were used, *A*, a very light soil with under 15 per cent. of clay and *B*, a heavy clay soil (clay content over 30 per cent.). About fifty 23 cm. pots were filled with each type of soil and each block was divided into two, so that there were four blocks of plants: *A* 1 and *A* 2 with light soil and *B* 1 and *B* 2 with clay soil. The hygroscopic moisture and the loss on ignition (organic content) for the *A* soil were 3·62 per cent. and 17·05 per cent. respectively, while for the *B* type of soil they were 2·61 per cent. and 14·58 per cent.

The blocks of plants were arranged with *A* 1 and *B* 2 on one side and *B* 1 and *A* 2 on the other side of the glass-house. The shortest distance between any two of the blocks was 120 cm.

Each pot received 800 c.c. of water per week, and it was found that with this amount of water *A* hardly formed surface cakes, while *B* formed a very hard resistant crust on the surface. Blocks *A* 2 and *B* 2 had the soil in the pots tilled (*i.e.* broken up with a fork) after each watering, so that the soil in block *B* 2 was kept in a loose condition in spite of its tendency to cake.

The mean temperature for the period of the experiments was 19° C. (max. 30·9° C., min. 7·2° C.) and the mean humidity for the same period

was 73·5 per cent., so that these conditions were very favourable for thrips development.

As in previous years, the plants were almost free from insects other than thrips, except at the end of the season when a number of plants, noticeably on blocks *B* 1 and *B* 2, became infested by an aphid (*Macrosiphum gei*) and for about a fortnight in the middle of August when the glass-house was invaded by large numbers of a noctuid caterpillar (*Plusia gamma*).

Infestation counts of thrips were made at intervals of a few days—on the average every four days.

At the end of the period of the experiments, the mean larval infestation factors for the four blocks were:

Untilled:	*A* 1, 24·2	larval thrips per 100 sq. cm. of leaf surface
	B 1, 21·5	,, ,, ,, ,, ,,
Tilled:	*A* 2, 31	,, ,, ,, ,, ,,
	B 2, 28·8	,, ,, ,, ,, ,,

That is, the least infested block for the whole season was *B* 1 with 21·5 larval thrips per 100 sq. cm. of leaf surface; this was the block of plants grown in untilled clay soil, while *A* 2, the block with light, tilled soil was the most infested of the four blocks. The difference between the infestation of *A* 1 and *A* 2 was almost the same as the difference between *B* 1 and *B* 2, but the average infestation of the two blocks in light soil was slightly higher than the average of the two clay soil blocks, though *B* 2 (tilled clay soil) had a higher infestation factor than *A* 1 (untilled light soil).

These results suggest that the looser soil is more favourable to thrips development than heavy soil which easily forms surface crusts, but if the graphs of the infestation counts are compared the difference between the two types of soil becomes much more striking. Fig. 1 is a graph giving the average larval infestation factor at each count for *A* 1 and *A* 2 compared with the average infestation factors for *B* 1 and *B* 2. At the beginning of the season the thrips were a little more numerous on the *B* blocks than on the *A* blocks, but about the middle of the counts the numbers of thrips on the *A* blocks began to increase rapidly, and in the latter part of the experiments the infestation factors for the blocks of plants in light soil far exceeded the factors for *B* 1 and *B* 2. It is probable that the counts at the beginning of the season are not really representative, as at the beginning of the experiments the thrips are more or less evenly distributed about the glass-house and, for at least the first generation, have

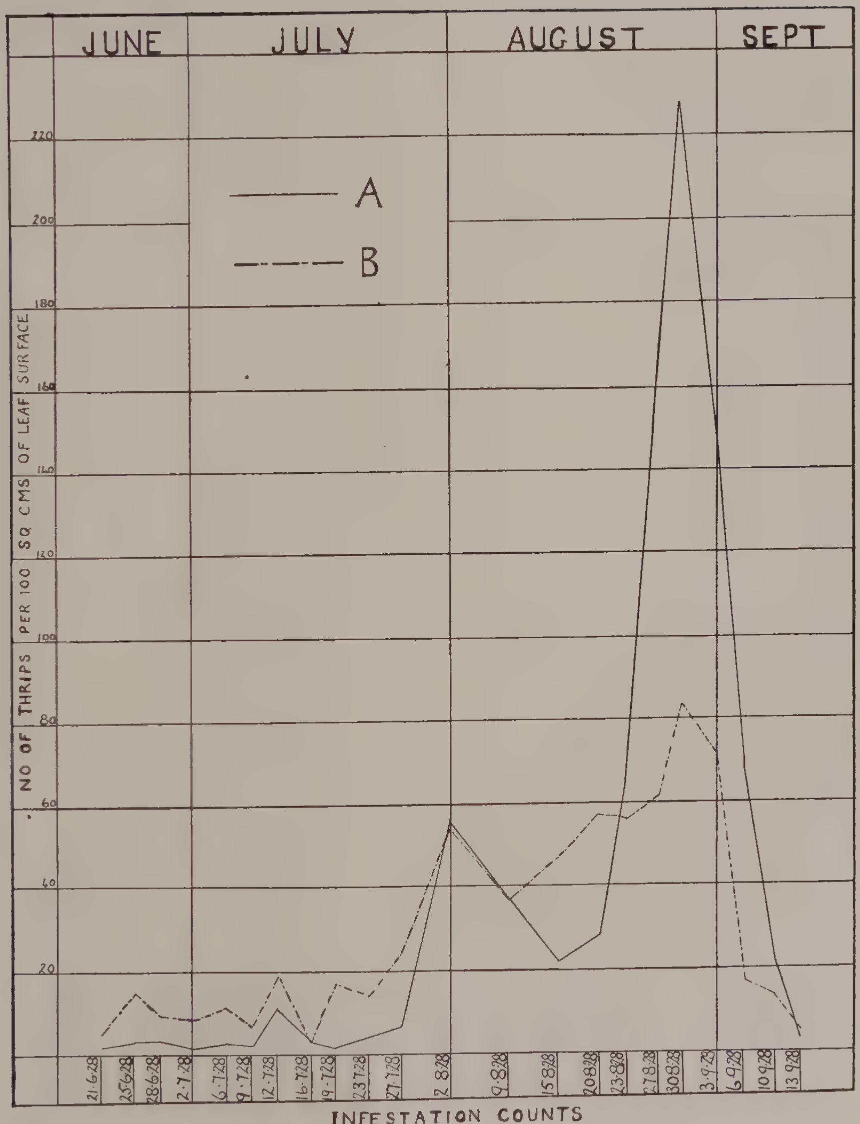

Fig. 1. The average larval infestation of the two *A* (light soil) blocks of plants compared with the average larval infestation of the two *B* (clay soil) blocks of plants.

no correlation with the soil in the pots. The plants in the *A* blocks also, although they were better grown and appeared healthier than the other blocks, were not so resistant to sudden changes in temperature as the plants in clay soil which had fewer and smaller leaves of a more xero-

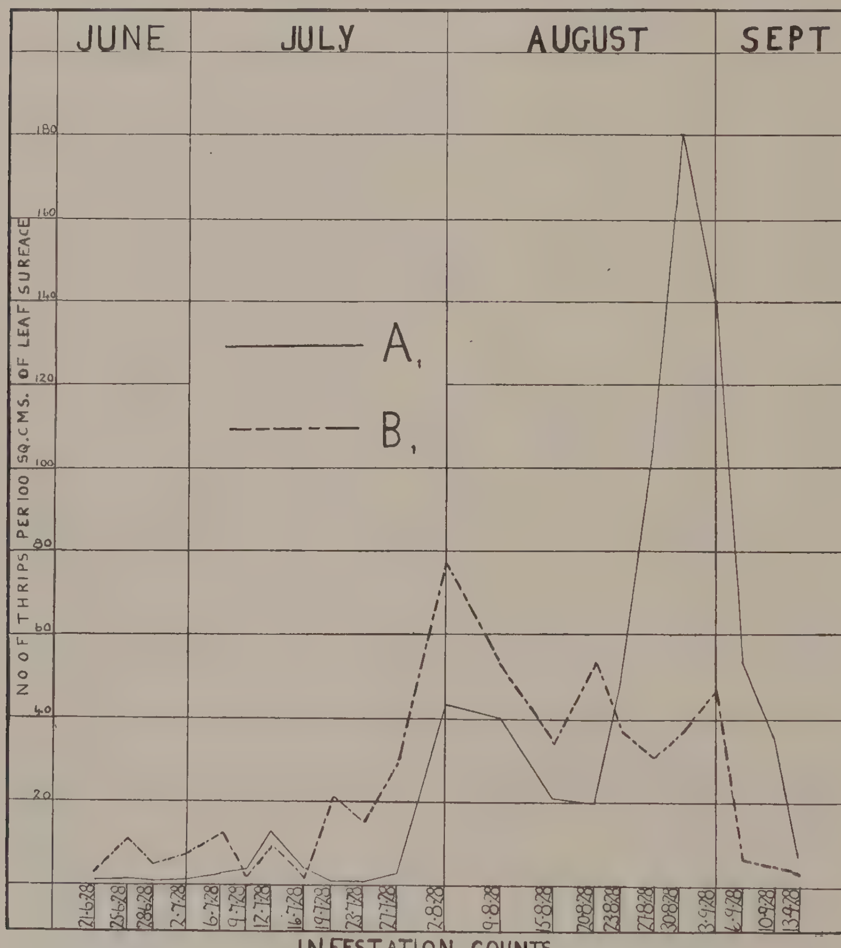

Fig. 2. The larval infestation of block *A* 1 (untilled, light soil) compared with the larval infestation of block *B* 1 (untilled, clay soil).

phytic type, and twice during the early part of the experiments there were sudden great rises in temperature and many of the leaves on the *A* plants were partially withered or scorched and numbers of thrips on these plants were killed, apparently by the heat, so that in this way the

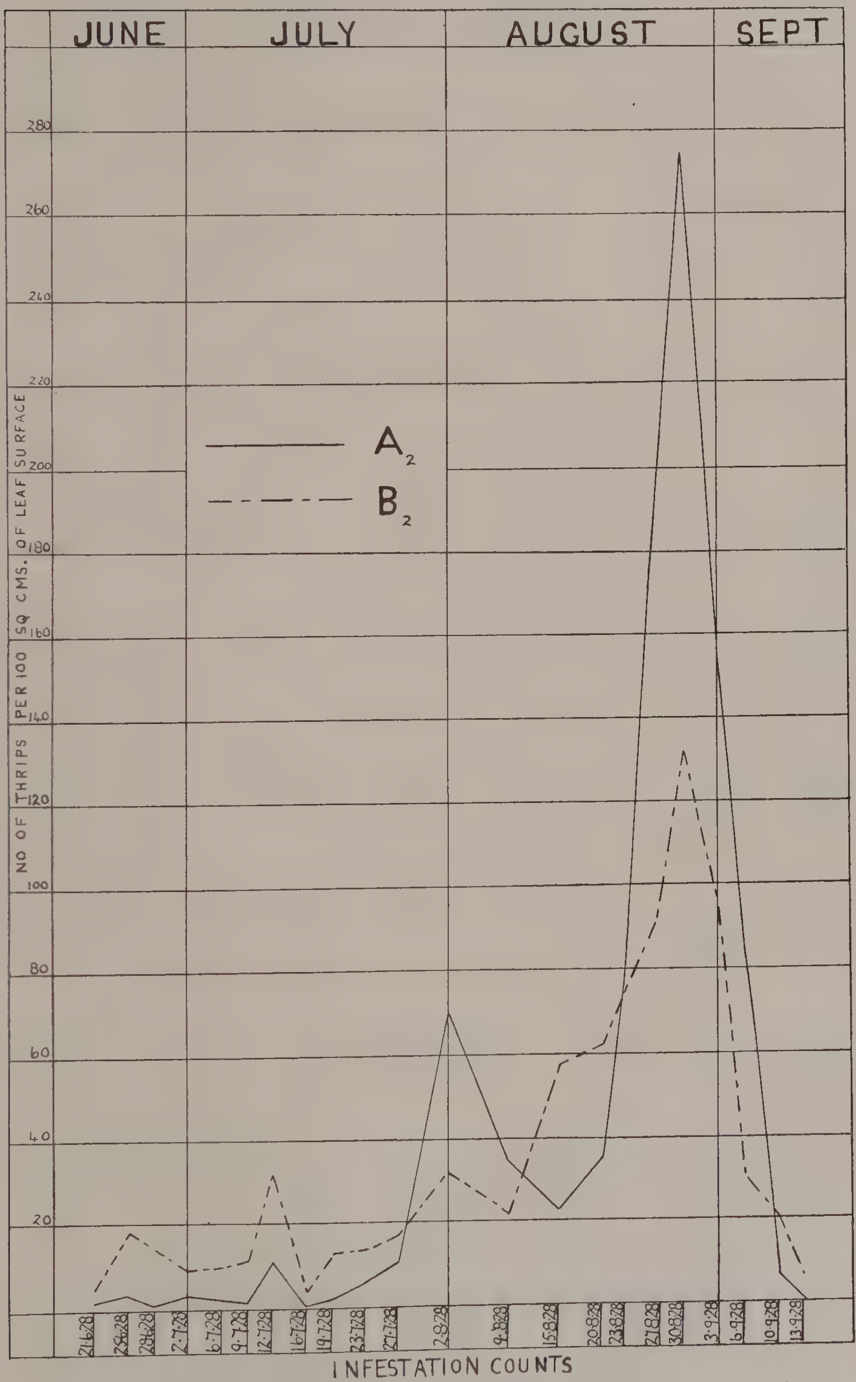

Fig. 3. The larval infestation of block *A* 2 (tilled, light soil) compared with the larval infestation of block *B* 2 (tilled, clay soil).

infestation of the two blocks of plants in light soil received checks by conditions which did not seem to affect the insects on the plants in clay soil.

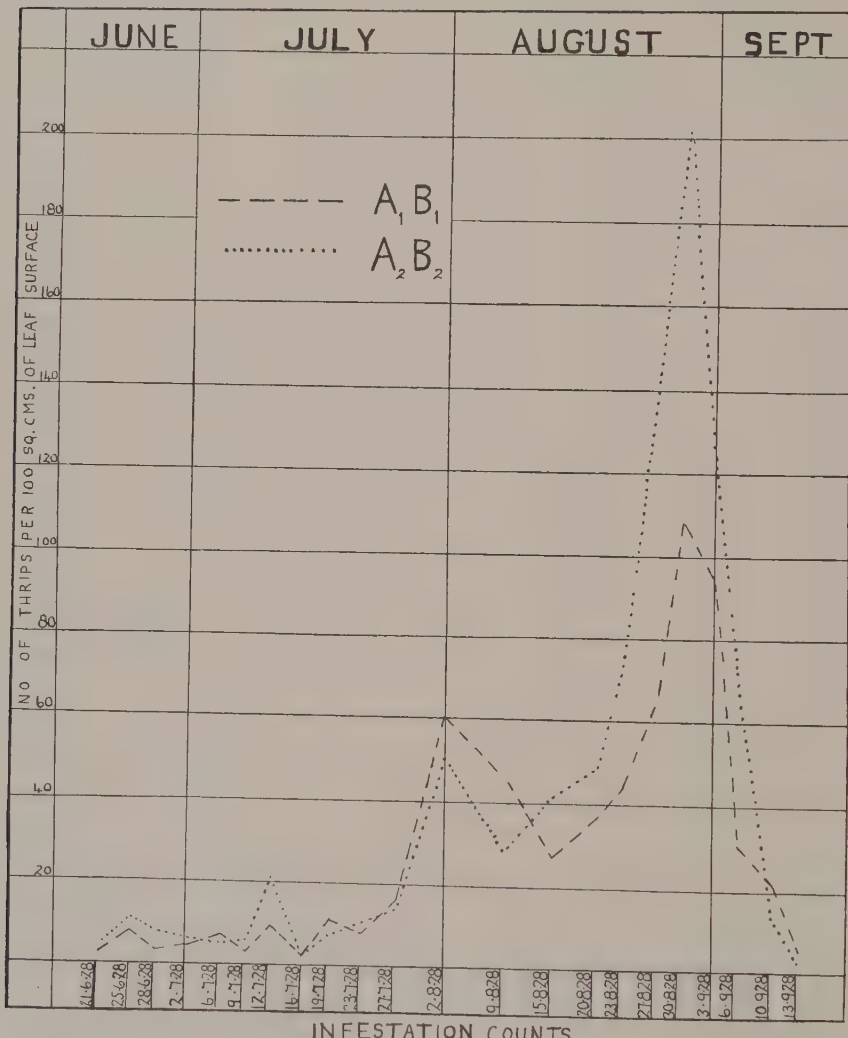

Fig. 4. The average larval infestation of blocks *A* 1 and *B* 1 (untilled soil) compared with the average larval infestation of block *A* 2 and *B* 2 (tilled soil).

The numbers of thrips on the plants gradually increased until a maximum was reached at the end of August. That is to say, on three of the blocks the numbers continued to increase until the end of August,

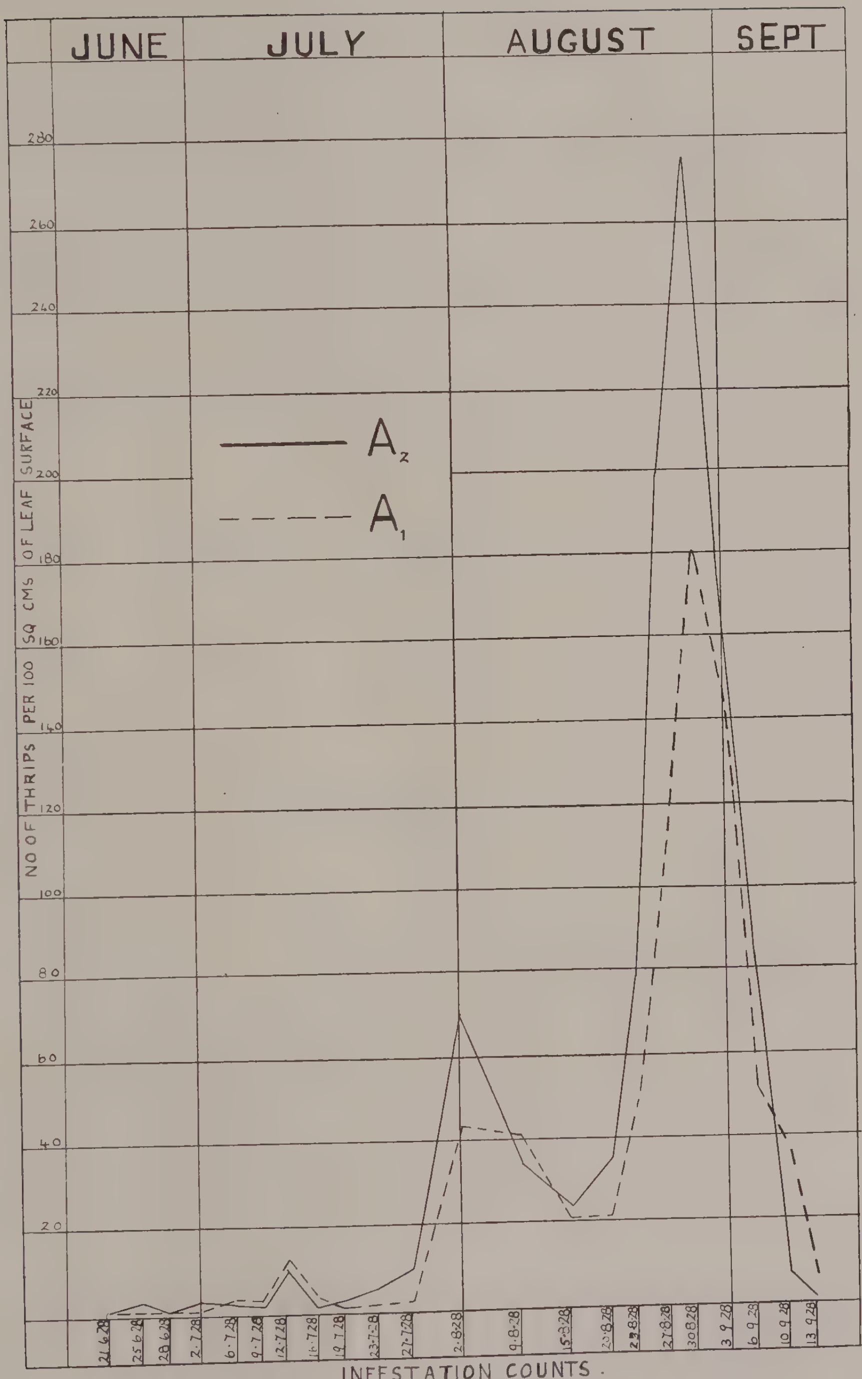

Fig. 5. The larval infestation of block *A* 1 (untilled, light soil) compared with the larval infestation of block *A* 2 (tilled, light soil).

but on block *B* 1 the maximum number of larval thrips occurred nearly a month earlier than on any of the other three blocks. The highest average larval infestation factor obtained for the plants in clay soil was 82·5 per 100 sq. cm. of leaf, while for the plants in light soil 227 larval thrips per 100 sq. cm. of leaf surface were counted.

The second figure gives a comparison of the counts for the two blocks with untilled soil, *A* 1 and *B* 1; this graph corresponds closely with Fig. 1 except that the maximum number of larval thrips for *B* 1 occurs earlier

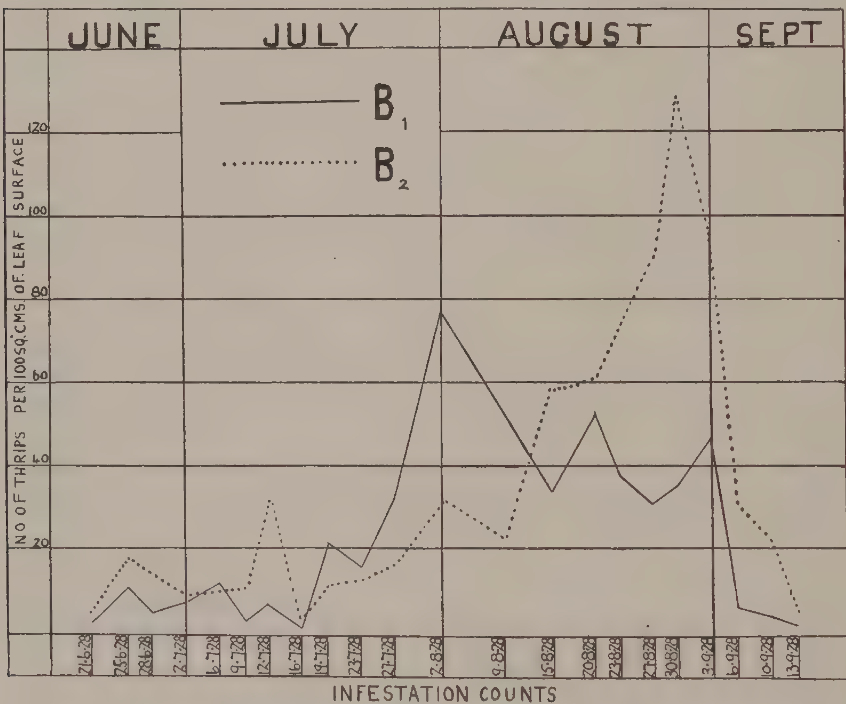

Fig. 6. The larval infestation of block *B* 1 (untilled, clay soil) compared with the larval infestation of block *B* 2 (tilled, clay soil).

in the season than the maximum number on *A* 1. The maxima for these two blocks were:

A 1, 180 larval thrips per 100 sq. cm. of leaf surface
B 1, 77 ,, ,, ,, ,, ,,

The infestation of the two blocks with tilled soil is compared in Fig. 3. The maximum numbers of larvae occurred in both cases at the end of August. The numbers were:

A 2, 274 larvae per 100 sq. cm. of leaf surface
B 2, 130 ,, ,, ,, ,,

so that in both tilled and untilled soil the thrips reach a much higher maximum on plants grown in light soil.

Fig. 4 gives a comparison of the average infestation of A 1 and B 1 with the average infestation of A 2 and B 2; this again shows clearly the

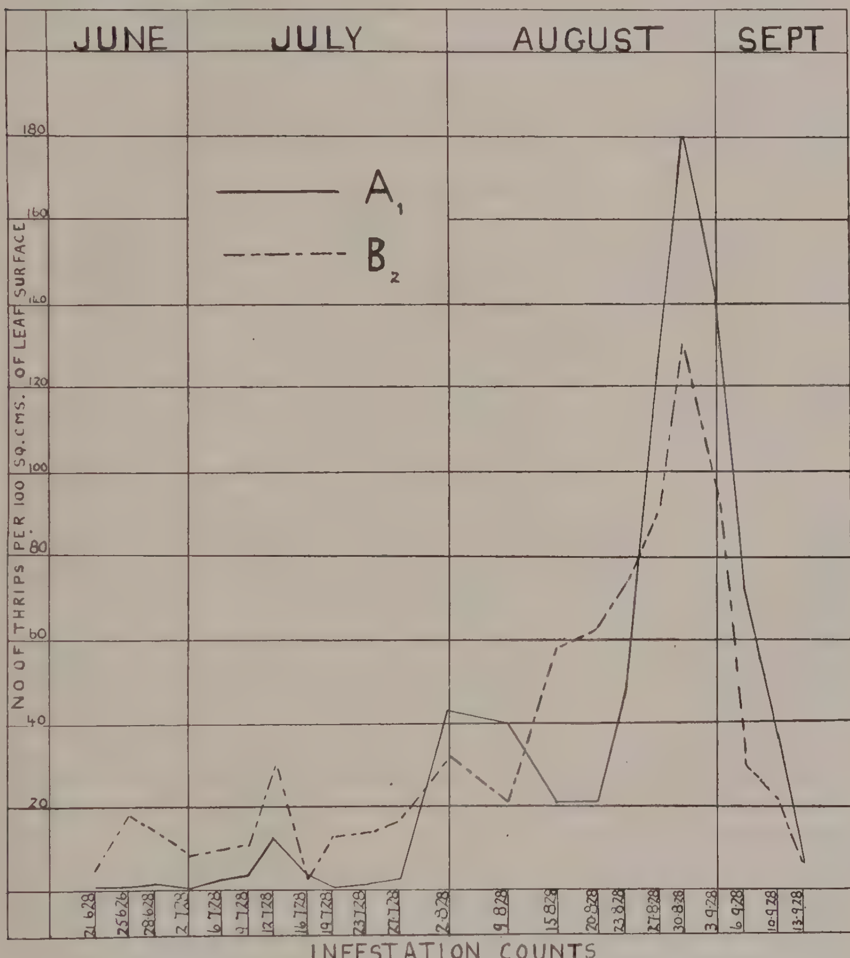

Fig. 7. The larval infestation of block A 1 (untilled, light soil) compared with the larval infestation of block B 2 (tilled, clay soil).

greater percentage of thrips on plants grown in tilled soil compared with plants in untilled soil; while Fig. 5 (A 1 compared with A 2) and Fig. 6 (B 1 compared with B 2) emphasize this point, as even in the light soil which only formed very slight surface crusts, tilling tends to increase the amount of infestation.

In the last graph (Fig. 7) the infestation of block *B* 2 (tilled clay soil) is compared with the infestation of block *A* 1 (untilled light soil); this shows that while the average infestation factor for the whole season is a little higher for *B* 2, the infestation never reached quite such a high level on this block as on *A* 1; this may be explained by the fact that, particularly during the hottest part of the season, the clay soil after being tilled had a tendency to dry into lumps, each of which may have imprisoned numbers of thrips, whereas the light soil with the higher temperature crumbled more and more readily and so approached more nearly the tilled condition.

Conclusion.

It seems clear from these experiments that light, non-caking soil is more favourable for the infestation of plants by *Thrips tabaci* than heavy clay soil, and these results are in agreement with the opinions expressed by the majority of authors. Moulton's paper on the orange thrips is the only one dealing with a soil pupating species in which it is suggested that clay soil is a factor in favour of thrips multiplication, and his results are not confirmed by later workers on the same insect.

Cameron and Treherne, writing on the pear thrips, a species which has only one generation per year, think that the breaking up of the soil by tilling may cause it to warm up more readily and therefore encourage the emergence of the adult thrips; but apart from this suggestion there has been little attempt to explain why a light or well tilled soil is more suitable for thrips than clay soil. It seems probable that in soils which form hard surface crusts the newly emerged adult thrips find it difficult or impossible to force their way through the tightly packed soil particles so that large numbers of them perish in the soil, whereas in loose soil or even in clay soil which has become dry enough for cracks and fissures to appear in the surface crust, the thrips have less difficulty in finding their way out of the soil and on to the plant. It might be thought that the effect of soil conditions on the plant would be the important factor, but in an experiment given in an earlier paper (8), more than three times as many adult thrips emerged from pots in which the soil was kept loose and dry as from pots from the same blocks of plants in which the soil was kept damp and caked on the surface. It is also possible that a certain number of pupae may be killed by the cohesion together of the soil particles during the formation of the surface crusts.

I should like to express my gratitude to Prof. Dunkerly for his helpful criticism, and to Miss R. M. Smith for her assistance in making the infestation counts, and to Dr A. Smith for his analysis of the soils used.

SUMMARY.

1. In several previous papers on Thysanoptera it has been shown that the condition or type of soil may have some influence on the infestation of a plant by thrips.

2. Experiments were carried out to determine the effect of different types of soil on the infestation of the cotton plant by *Thrips tabaci*.

3. The types of soil used in the experiments were a heavy clay soil and a light soil with less than 15 per cent. of clay, about fifty pots being filled with each type.

4. Each block of fifty plants was divided into two and the soil in one half was tilled while in the other it was left undisturbed.

5. It was found that the plants in the block with untilled clay soil were least infested by *T. tabaci*, while the plants in the block with light, tilled soil were the most highly infested.

6. The average infestation of the two blocks with light soil was slightly higher than the average infestation of the clay soil blocks, in spite of the fact that at the beginning of the season the former blocks of plants and the thrips on them suffered severely from a sudden great rise in temperature.

7. In both types of soil the block with tilled soil was more highly infested than the other block.

8. It appears from these experiments that light soil is more favourable to the multiplication of soil pupating species of thrips than an easily caking, clay soil and that tilling the soil increases the infestation by these insects.

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THIONIN AND ORANGE G FOR THE DIFFERENTIAL STAINING OF BACTERIA AND FUNGI IN PLANT TISSUES

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(With Plate VII.)

In the course of studies on the disease of cotton caused by the organism *Bacterium malvacearum* the need arose for a method of tracing the progress of the organisms through the tissues of the host. Sections were stained by many different methods, most of the well-known combinations, such as the Pianeze stain as used by Vaughan (1), the Giemsa stain as modified by Wright and Skoric (2), Ziehl's carbol-fuchsin and light green, iron alum haematoxylin and so on, being tried, as well as a number of other combinations. None, however, gave a really satisfactory result.

The organism produces a considerable amount of slime, and this stains very readily, with the result that the bacteria are obscured by the diffuse stain. Further, none of the combinations referred to differentiated between the slime and the host tissues.

Thionin is well known as a stain for differentiating mucin in animal tissues owing to its high metachromasy, mucin being stained pink and other tissues shades of blue and purple. Used in aqueous or phenol solution on diseased plant tissue it gave very promising results, but the required degree of differentiation of host and parasite was not obtained owing to the intense staining of the host tissue. Orange G in alcoholic solution was, however, found to be a good differentiating agent and at the same time acted as an excellent counter-stain for the cellulose walls. The technique adopted was as follows:

Paraffin sections.

(1) Xylol to remove wax.

(2) Grade through alcohols to water.

(3) Stain in the following solution 1 hour: thionin, 0·1 gm.; 5 per cent. solution of phenol in distilled water 100 c.c.

(4) Grade through alcohols to absolute alcohol.

(5) Differentiate in a saturated solution of orange G in absolute alcohol.

(6) Wash thoroughly in absolute alcohol.

(7) Xylol-alcohol.

(8) Xylol.

(9) Mount in balsam.

The differentiation is accomplished fairly quickly, usually in about ½ to 1 minute. The progress may be controlled under the microscope, but with a little practice satisfactory differentiation can be carried out by eye observation only. The treatment with orange G is continued until the sections lose their bluish-purple colour and become uniformly yellowish green.

In plant tissues the parasite is stained violet-purple, cellulose walls yellow or green, lignified tissue blue, nuclei pale blue with purple nucleoli and chromosomes in dividing nuclei deep blue on a purple spindle. Nuclei in fungal hyphae are clearly picked out in deep purple. For hand sections the procedure may be shorter.

(1) Sections in water.

(2) Stain in carbol-thionin 5 minutes.

(3) Wash in water.

(4) 95 per cent. alcohol.

(5) Differentiate in the solution of orange G (several minutes).

(6) Wash well in absolute alcohol.

(7) Clear in xylol.

(8) Mount in balsam.

The stain has been found to give good results with such different materials as *Bacterium malvacearum* on *Gossypium*, *B. radicicola* in root nodules of legumes, *Plasmodiophora* on *Brassica*, *Synchytrium endobioticum* on *Solanum*, *Peronospora* on *Capsella*, *Phytophthora* and *Sclerotinia* on seedlings, *Botrytis* on *Allium*, and *Puccinia* on *Anemone*. The procedure is so rapid and so easily carried out that the stain combination should prove of value for class purposes.

Apparently any reliable brand of thionin is satisfactory; good results have been obtained with a sample from British Drug Houses and also with the "Soloid" brand tabloids of Messrs Burroughs, Wellcome and Co.

A modification which may prove of value in particular cases is to remove the orange stain by regrading the sections to water after differentiation, and then running up again to xylol. By this means all the orange is removed, leaving the parasite very conspicuous against unstained walls.

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EXPLANATION OF PLATE VII.

Fig. 1. *Bacterium malvacearum* in stem of cotton plant. Microtome section of material fixed in Zenker's fluid. Stained thionin and orange G. Photographed with $\frac{1}{6}''$ achromatic obj. $\times 10$ Periplanatic ocular. Panchromatic plate. Wratten "M" filter (green) $\times 530$.

Fig. 2. *Peronospora parasitica* in stem of *Capsella*. Hand section of old material in spirit. Stained thionin and orange G. Photographed with $\frac{2}{3}''$ achromatic obj. $\times 10$ Periplanatic ocular. Panchromatic plate. Wratten "M" filter (green) $\times 100$. Note the differentiation of nuclei in the fungal hyphae.

(*Received October* 18*th*, 1929.)

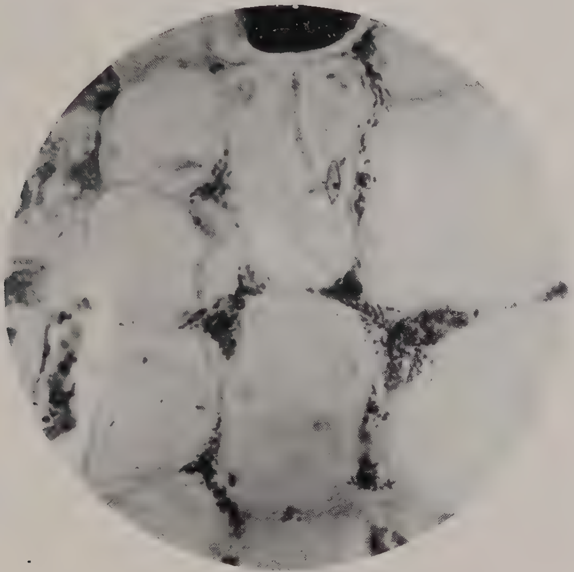

Fig. 1.

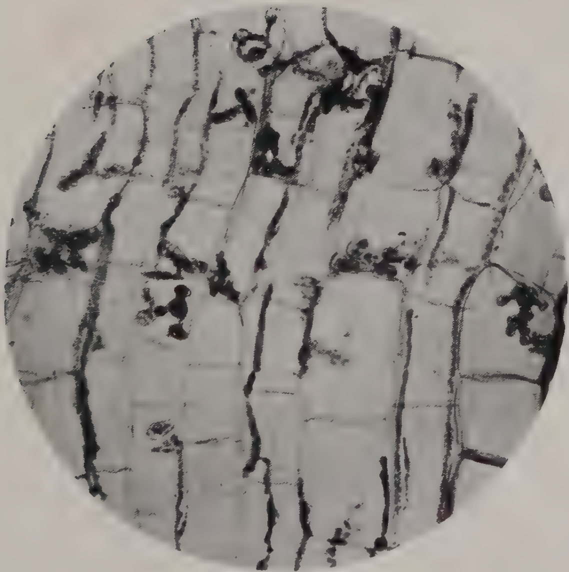

Fig. 2.

STOUGHTON.—THIONIN AND ORANGE G FOR THE DIFFERENTIAL STAINING OF BACTERIA AND FUNGI IN PLANT TISSUES (pp. 162–164).

PROCEEDINGS OF THE ASSOCIATION OF ECONOMIC BIOLOGISTS

Ordinary Meeting held at 2.30 p.m. on Friday, October 25th, 1929, in the Botanical Lecture Theatre of the Imperial College of Science and Technology, London. The Chair was taken by the President, Dr E. J. Butler, C.I.E., F.R.S.

THE SCOPE OF THE WORK OF A RESEARCH INSTITUTE IN DAIRYING.

By Prof. R. Stenhouse Williams, M.B., D.Sc., D.P.H., and Members of the Staff of the National Institute for Research in Dairying, Shinfield, Reading.

I. INTRODUCTION.

By Prof. R. Stenhouse Williams, M.B., D.Sc., D.P.H.

Dr Stenhouse Williams gave a short account of the work of the National Institute for Research in Dairying, pointing out that the Institute had grown from very small beginnings and that as time had gone on it had become more and more possible for the members of the staff to visualise the field of their work which he outlined as follows.

If a Research Institute in Dairying is to fulfil its functions it must be prepared to demonstrate the exact influences of many factors upon the constitution of milk, and its resulting suitability for the preparation of a great variety of dairy products. The staff of the Institute are, therefore, of opinion that it is necessary to be in a position to study the constituents of the foodstuffs of the cow and their influence upon the milk which is secreted; such work clearly necessitates very detailed investigations of the final constitution of the milk and of the physiological processes which have led to that constitution.

When the milk has been obtained it is necessary to consider its value either as whole milk, or for the preparation of dairy products. The first of these studies is peculiarly vital in a country like England with its great urban population which consumes as milk about 50 per cent. of the total milk supply. Such a situation necessitates very careful studies of the methods of keeping and carriage of milk in the interests of the industry and the consumer. The second involves prolonged investigations into the best methods of preparation of the different types of dairy products for two reasons: (*a*) A prosperous dairying industry in England can only be ensured by maintaining a very high standard of quality. In the lower grades we cannot compete with cheaper sources of supply. (*b*) The maintenance of a high quality of goods automatically eliminates many of the faults which at present exist and the need for direct monetary loss and to diminished sales.

It is clear that studies of the preparation of dairy products must include investigations concerning the uses which can be made of the by-products, particularly whey and separated milk. There is good reason to believe that these can be employed much more usefully than is sometimes the case at present.

In relation to the above problems many other types of investigation are required; as, for example, the study of the efficiency of dairy appliances for the work for which they are designed. A considerable amount of work of this character has been carried out at the Institute and has led to material improvements being made in some types of dairy appliances.

Anyone who gives thought to the field of work which has been outlined, and considers what is involved in the efficient study of any one of these problems, must realise the magnitude of the task upon which we are engaged. We ourselves realise it, but are glad that time and work have enabled us, as we think, to obtain a clear conception of our duties. This fact is of great value to us in our efforts to develop the work of the Institute and make it worthy of the industry which its serves.

II. A NOTE ON THE ECONOMIC ASPECTS OF THE PASTEURISATION OF MILK FOR CHEESE MAKING.

By G. M. Moir, M.Sc.

The pasteurisation of cream to be used for the manufacture of butter has long been a feature of dairy factory practice. The success of this procedure naturally led to attempts to do the same thing with milk for cheese making when difficulties were encountered with its hygienic quality. In spite of the fact that the heating of the milk produces in it certain slight but definite chemical changes, notably a decreased coagulability with rennin, good Cheddar cheese can be made from pasteurised milk, provided that it is not overheated. This has been repeatedly demonstrated in carefully controlled experiments conducted by workers in different countries, and subsequently confirmed by extensive use in many cheese factories.

The economic advantages of the procedure are:

(1) Slightly larger yield of cheese, which is chiefly due to the incorporation of more moisture.

(2) Loss of less fat in the whey, thereby reducing the factory's output of whey butter which is usually an inferior product.

(3) But chiefly the ability of the cheese maker to produce regularly every day cheese of a uniform quality unspoiled by bad flavours originating from excessive bacterial content of the milk supply.

These advantages are slightly offset by the cost of the energy required to heat and cool the milk and also by the longer time of ripening required by cheese from pasteurised milk. Nevertheless, pasteurisation has been found a great help in Cheddar cheese making, for by eliminating the few bad cheeses and generally raising the level of the product it has created confidence among the buyers and distributors, which has been reflected in steadier prices for the primary producers.

For the factory manager the process of cheese making has been simplified and brought more under control so that the curd in its various stages does not require the

careful watching and manipulation that is necessary when no steps have been taken to reduce the large and varied bacterial flora possessed by the original milk. On the other hand, the factory manager with pasteurisation at his disposal tended to pay less attention to the bacteriological quality of his milk supply. When in hot weather this gave trouble he would probably attempt to remedy it by pasteurising at a slightly higher temperature. Now there is ample evidence available that if the milk is overheated the resulting cheese suffers considerably in texture, for the curd will not mat together properly. In addition the development of the mature tasty flavour expected of a Cheddar cheese is much inhibited. Both these factors depreciate the market value of the cheese, and both are to a large extent due to chemical changes about which little is known.

Thus although pasteurisation of milk for cheese making may be of great benefit to the industry yet in the end it may intensify defects. This is because it is a method of attacking a problem halfway along the line instead of starting at the beginning. Pasteurisation may prove a very good stopgap, but when the fundamental problem —the improvement of the bacteriological content of the milk supply—has received due attention it is quite likely that it will be possible to make the best cheese from raw milk that has been obtained under satisfactory hygienic conditions.

III. FISHINESS IN DAIRY PRODUCTS.

By W. L. Davies, Ph.D., F.I.C.

Owing to its physical properties, the fatty material of dairy products is much more resistant to breakdown than the protein and carbohydrate constituents, but, under certain conditions, even the fatty material can undergo deterioration yielding various taints and flavours in the product. It is proposed here to outline the nature of these degradations and the conditions causing them.

Auto-oxidation of fat.

The ethylenic linkage (—C = C—) of oleic acid and the multiplicated linkages of the more unsaturated fatty acids of fats can take up oxygen to form peroxides and these compounds either split at the original double bonds to form various aldehydes or liberate, by interaction with water, active oxygen (as hydrogen peroxide). More oxygen is taken in and the process continues until the aldehyde concentration is sufficient to confer a tallowy taste and odour to the fat. This, in principle, is the mechanism of the breakdown of fat by auto-oxidation.

Pure fats, nevertheless, will not show any appreciable oxygen uptake for a considerable length of time, after which, once the absorption commences, the uptake increases in speed at an increasing rate, showing the oxygen absorption to be autocatalytic. The period of time necessary before the appreciable oxygen uptake commences is termed the "induction period" and is that length of time during which fat, in contact with air, remains in a wholesome condition. The length of this period of induction, therefore, represents comparatively the keeping quality of the fat. Again, conditions tending to shorten this period will tend to lower the keeping quality of the fat. The period of induction is appreciably shortened by the following factors: (*a*) exposure to heat and light, both of these factors adding energy to the system; (*b*) acidity,

in that free acidity is usually represented by free unsaturated acid which is more reactive in the free than in the combined state; (*c*) activators of molecular oxygen, such as traces of heavy metals, e.g. copper, iron, nickel or manganese, or ultra-violet light. In brief, the period of induction is shortened by all conditions favouring a high oxygen potential of the system, and by "oxygen potential" is meant the concentration of activated oxygen, on which the rate of oxidation depends. Of the above factors, traces of the heavy metals mentioned are by far the most potent in bringing about the oxidation, copper being the most virulent. The above factors also catalyse the actual oxidation, when the period of induction is over.

The fat in milk products.

The fat of dairy products is more reactive to the above actions owing to (*a*) the very fine state of division of the fat which entails the exposure of a very large area per unit weight of fat, (*b*) the presence of a hydrophilic colloid, such as lecithin, which acts as a sympathetic agent between the aqueous and fat phases. In this connection lactic acid and free fatty acids are of significance, as well as the inorganic salt content of the product in which lecithin is slightly soluble. In butter, where the emulsion has been converted from the oil-in-water to the water-in-oil type, the condition of high area exposure is still maintained. Lecithin also is a special sort of fat-like substance which is more reactive than fat proper and, therefore, where conditions are such that fat will tend to deteriorate, it is the lecithin which will first break down. In lecithin, one of the groups attached to the glyceryl radical is a phosphoric acid choline complex and, under conditions of oxidation this complex is attacked and the choline is oxidised to give trimethylamine:

$$—CH_2.O.PO(OH)_2—C_2H_4.N(CH_3)_3OH \rightarrow —CH_2.O.PO(OH)_3 + N(CH_3)_3 + 2CO_2 + 2H_2O.$$

The breakdown of choline is similar to the breakdown of amino acids to yield free ammonia by Fenton's reagent (hydrogen peroxide in the presence of ferrous salts). The presence of free trimethylamine formed in this manner confers on the product a pronounced fishy taste and smell, and the above mechanism of the reaction explains the source of the causative ingredient.

The breakdown of the lecithin thus occurs first but is followed immediately by the oxidation of the proper fat which gives rise to the form of rancidity known as "tallowiness." Under very strong catalytic action to oxidation the "fishy" taint is hardly observable, being submerged by the quick development of the tallowy taint, but with slowly catalysed oxidation the fishy period is longer and better detected.

The occurrence of "fishiness" in stored butters is thus partly ascribed to the presence of metallic contamination of the butter by small traces of metals dissolved off the factory equipment during the collecting and processing of the cream and its churning into butter. The use of properly tinned or otherwise non-corrosive equipment would minimise the appearance of this fault in stored butters.

Other factors governing fat deterioration.

As mentioned above, the principle underlying fat oxidation is the maintenance in the system under consideration of a high level of oxygen potential which is maintained in the prominent cases by the activating properties of the catalysts. The growth of micro-organisms in the above systems, on the other hand, endeavours to maintain the

system at a constant but much lower oxygen potential, and if conditions are such that micro-organisms can thrive, no fat oxidation can occur. With butter, storage at low temperatures minimises micro-organic activity and although the rate of chemical oxidation is suppressed by low temperature, the sum total of the effect is to favour slightly chemical oxidation. The potency of the catalyst is also of significance in that it determines in which direction the balance of the oxygen requirement for chemical oxidation as against the requirement of the actively growing organism for oxygen will turn. The catalyst maintains a certain concentration of activated oxygen. If the concentration of catalase of the growing organism in the system is high enough to deactivate this toxic constituent, the balance turns in favour of the organism, whereas if the catalase concentration is low, the organism succumbs to the toxic effects of (to that organism) an abnormally high activated oxygen content. (It thus appears that where small traces of copper salts are used to cope with the ravages of an organism, the toxic factor is the maintenance of a higher concentration of active oxygen, such as hydrogen peroxide, than the organisms can cope with.)

Finally, moisture conditions are of importance especially where dried-milk products are concerned. The more processing which a milk product has undergone the more is the amount of metallic contamination. The rendering of a medium more unfavourable to micro-organic growth by drying to a fine powder enhances the possibilities of chemical fat deterioration through the agencies of a possible increasing amount of copper and iron dissolved from the drying plant. The main step to take in order to avoid fat deterioration is to minimise contamination with the most potent catalysts to oxidation—the products of metallic corrosion.

IV. THE CULTURAL CHARACTERISTICS AND METABOLISM OF THE ORGANISM CAUSING "RED SPOT" IN ENGLISH HARD CHEESE.

By J. G. Davis, M.Sc., and A. T. R. Mattick, B.Sc.

"Red spot" or "rusty spot" occurs at irregular intervals in most English hard cheese made from April to September. The spots are small and discrete and seldom exceed a millimetre in diameter, the colour varying from a pale orange to a bright carmine red. The fault occurs throughout the cheese mass and especially in moist pockets at those fractures corresponding to the original pieces of curd. The spots appear within a few (3–6) weeks of manufacture. If a freshly cut surface of faulty cheese be exposed to the air the colour of the spots fades considerably in 24 hours.

"Red spots" are practically pure cultures of a small bacterium. Young cultures are Gram-positive, old cultures show Gram-negative and diphtheroid forms. The organism is non-motile, non-capsulated and non-sporing. It is not acid-fast. Growth is very slight except under anaerobic or micro-aerophilic conditions. The optimum temperature for growth is about 30° C. and for pigmentation about 20° C.

The optimum *p*H is from 5 to 7. Acid, but not gas, is formed in fructose, dextrose, galactose, and maltose peptone water. Lactose and dextrin are sometimes fermented. Proteins are not hydrolysed. Sugars are fermented to lactic and (from 12 to 50 per cent.) acetic acids.

A definite sharp acid aroma is characteristic of old cultures.

Apart from its pigment this organism would be classified as a lactic acid organism. The ability to produce pigment anaerobically sharply distinguishes it from the usual chromogenic bacteria which require oxygen for pigment production and reduce their pigment to a leucobase if air is withdrawn.

Nothing is known of the nature or function of anaerobic pigments.

The pigment of this organism is only produced in stab cultures or rarely and with difficulty by surface cultures under anaerobic conditions. Its production is stimulated by (1) moisture, (2) reduced O_2 tension, (3) an interface.

A fermentable carbohydrate and some "factor," present in yeast and some peptones, but not in meat extract and other peptones, are essential for pigmentation. Pigment production is stimulated by high soluble-N concentrations, but the pigment factor is not any known amino acid or a cleavage product of pure casein. It is present in milk curd however, and in most plant and animal tissues.

	Growth	Pigment
Beer wort	+ + +	+ + +
Milk (slow)	+ +	+ + +
Fruit juices	+ + to + + +	+ to + + +
Heart infusion	+ +	+ + +
Animal tissue extracts	+ +	+ + to + + +
Oat and wheat extract	+ +	+ + +

Pigmentation is apparently not dependent on traces of metals (cf. Mg for *B. prodigiosus*).

The distribution and properties of this "factor" have been found to resemble closely those of the vitamin B complex. At the time of writing, no substance deficient in this complex has been found to contain the pigment factor and all substances containing the complex enable the bacterium to produce pigment.

	B_1	B_2	"Bios"	Growth	Pigment
Yeast	+	+	+	+ + +	+ + +
Honey	–	–	–	–	–
Egg white	–	+	?	–	–
Egg yolk	+	+	?	+ + +	+ + +
Peter's B_1 concentrate	+	–	.	+ +	+ + +
Fibre	?	?	+ +	–	–

B_2 and "bios" are not identical with the "pigment factor" which is always associated with B_1.

No recognised chemical substance present in milk permitted pigment formation when added to a basal medium.

Chemical nature of pigment.

A living pigmented culture loses its colour rapidly on exposure to air. If killed by drying or by alcohol, the pigment is fixed.

It is insoluble in water, alcohol, ether, benzene, chloroform, glycerine, dilute acids and alkalies. Boiling with concentrated acids destroys the pigment; it is, however, stable to boiling alcoholic potash. A deep blue-green colour is obtained with concen-

trated H_2SO_4. If the pigment is a lipochrome as this colour reaction suggests, its general insolubility presents a difficult problem.

Note.

Further investigation of this fault will be published in the *Journal of Dairy Research.*

V. THE NEED FOR FURTHER KNOWLEDGE CONCERNING PARASITIC DISEASES OF PIGS.

By A. H. Blissett, B.Sc., and W. L. Little, F.R.C.V.S.

The work of the National Institute for Research in Dairying has included a number of nutritional problems which have involved the feeding of pigs. A suitable animal house has been erected to accommodate this branch of the work and has proved to be capable of accommodating about 100 pigs. As the needs of the work increased the breeding of stock pigs was transferred to the farm, where accommodation was found in woods and outlying buildings comparable with the conditions obtaining in a large number of farms. This arrangement appeared to be satisfactory at the start, but since 1926 we have experienced obscure ailments, sometimes resulting in death, among our pigs. We consulted Prof. Buxton of the Animal Pathology Department at Cambridge and after investigation came to the conclusion that the deaths were due to internal parasites.

We thought that our experiences in this matter might be of interest to Economic Biologists.

The results of the investigation of these earlier cases are reported in the *Pig Breeders' Annual*, 1926.

The first outbreak was attributed to an infection by *Hyostrongylus rubidus*, which, in conjunction with *coccidiosis*, was considered the probable cause of the thirteen deaths that occurred. At this time the source of infection had not been traced.

In the month of June this year two more pigs died and again we consulted the Animal Pathology Department at Cambridge. This time the pigs were found to be strongly infested with *Oesophogastomum dentatum*, which probably caused their deaths.

In order to endeavour to trace the origin of this trouble we started an examination of all the pigs on the farm for the presence or absence of the ova of nematodes. In this examination we used the method described by Sheather in the *Journal of Comparative Pathology and Therapeutics*, XXXVI, 1923, p. 71. The technique was as follows:

The faeces were emulsified with water until their motility approximated to that of water. They were then strained through wire gauze which had thirty meshes to the linear inch. A liquid portion of the strainings was mixed with an equal volume of sugar solution which was made by dissolving 1 lb. of sugar in $\frac{3}{4}$ pint of water. This suspension was centrifuged for $1\frac{1}{2}$ minutes at 2500 R.P.M. The eggs came to the surface and were fished out on a coverslip which was then examined under the microscope.

(1) Faeces were collected from all the sows running in the woods, and in every case infestation was noted to be very great. Examination of the boar's faeces showed a huge number of worm ova.

(2) Faeces were collected from all the sows in the farrowing pens in the old outlying buildings. Heavy infestation was again noted, as also was the case with the

faeces collected from ten newly weaned pigs running about in the yard attached to the old buildings.

During the same period we made periodical examinations of the faeces obtained from the pigs in the animal house, which is cleaned out daily and the sties are frequently limewashed and disinfected.

The examinations primarily took place of faeces collected from groups of pigs which had been in our animal house for varying periods on three different types of experiments.

(3) Two pens which had been in the piggeries for ten weeks. No presence of ova noted though two examinations were made.

(4) Two pens which had been in the piggeries for seven weeks. Ova were found to be present in the faeces of one pen only and on a subsequent examination a week later no worm ova were discovered.

(5) Four pens, including those in which the two deaths occurred. These were examined shortly after the diagnosis was received from Cambridge and all pens showed positive results.

After this first examination the faeces of the remaining six pigs from the two pens in which the two dead pigs were formerly situated were examined periodically with the following results: From the first examination four of the pigs were noted to be infested. On subsequent examinations two pigs only were noted with infestation which appeared to diminish as time went on.

(6) With suckling pigs difficulties were experienced in obtaining samples, but in the few cases examined ova were not found as a general rule in the faeces of very young pigs, but were invariably found when the pigs were nearing weaning stage.

It would appear from (5) that the longer the pigs are maintained in clean surroundings the more the infestation diminishes. This is also borne out by the examinations (3) and (4) where the pigs had been in the animal house for a considerable period and showed little or no infestation.

It appears from our experience that pigs can become infected at a very early age, especially when they are kept under dirty conditions.

It also appears that the conditions under which our breeding sows were kept were more favourable for parasitic infection than was the case in the animal house.

Further practical application of the knowledge of the life history of these parasites, more especially of the time and conditions which favour the change of the ova to the embryonic stage, is much needed.

VI. PROXIMATE CHEMICAL ANALYSES OF PASTURES FROM PLOTS MANURED WITH SULPHATE OF AMMONIA.

By J. Golding, D.S.O., F.I.C., and H. S. Hallett.

Capt. Golding gave a short account of the chemical work which is being carried out in connection with some experiments on manuring of pastures with sulphate of ammonia.

There are four plots on the farm, two of which are controls and two of which have been manured with sulphate of ammonia at intervals. The analytical work, which

commenced in 1926 and is still in progress, involves the chemical examination of samples of grass taken from all over the plot at the time at which the cows begin to graze each plot in turn. The samples represent as far as possible the grass which the cows actually graze. The outstanding feature which has emerged up to the present is the high protein content of the dry matter of the grass of the manured plots in the early spring and after the summer rains. The differences between the manured and unmanured plots are very marked at these periods of the year. Charts were shown exhibiting these differences from which the author did not feel that any final conclusions could be drawn as the work is still in progress.

VII. DISCUSSION.

By Dr Goodey.

In response to Mr Blissett's request for information on the life history of the nematode parasites of the pig and whether the eggs passed in the faeces are capable of infecting the host immediately after being voided, Dr Goodey gave a brief account of the larval life history as it occurs in *Ascaris lumbricoides, Hyostrongylus rubidus, Oesophagostomum dentatum* and *Metastrongylus apri.*

He explained that in the case of *Ascaris lumbricoides*, parasitic in the small intestine, the eggs are passed in a segmented condition and under favourable conditions of moisture and aeration continue to develop till an embryo is produced within the shell membranes. The latter are very resistant to comparatively strong acids, alkalis and solutions of salts and for laboratory purposes eggs are conveniently cultured in solutions of formalin of 2·5 per cent. strength. The eggs also cling tenaciously to comparatively smooth surfaces so that it is a very difficult matter to remove them from floors or walls of pigsties. Hot solutions of carbolic acid or other disinfectants of the creosote class are capable of destroying eggs in all stages of development.

When embryonated eggs are taken in by the host or by laboratory animals, they hatch in the small intestine and the liberated embryos, boring through the wall of the gut, make their way into the blood vessels and so getting into the portal circulation are taken to the liver. From this they reach the heart and thence are pumped to the lungs. Here they pass from the fine blood vessels into the alveoli and in so doing set up a condition of verminous pneumonia. They escape from the lungs *via* the bronchioles, bronchi and trachea, and are afterwards swallowed on reaching the throat. By this means they pass again through the stomach into the small intestine where they now grow to maturity.

Hyostrongylus rubidus is a small worm living on the mucosa of the stomach, where it sets up gastritis. The eggs of this species pass out in the droppings and on hatching give rise to small larvae which are not immediately infective but first feed and grow and after a time undergo a moult. They then undergo a further period of feeding and growth, after which they shed their cuticle and remain ensheathed within it, thus becoming infective larvae. Until this stage of development is attained they are incapable of infecting the host. The latter acquires the parasite by eating food accidentally contaminated by such infective larvae. Dr Goodey stated that he had worked out the above course of larval development and had published an account of it in the *Journal of Helminthology* a few years previously.

With regard to *Oesophagostomum dentatum*, parasitic in the caecum and colon, he stated that it does not cause nodules in the wall of the intestine but passes its life free in the lumen. It is wrong therefore to call it the "nodule" worm of swine. Its life history is very similar to that of *Hyostrongylus rubidus*. The eggs passed in the faeces hatch and give rise to larvae which require to feed and pass through a first moult followed by a further period of growth leading to the production of ensheathed infective larvae. Such infective larvae on being taken in by pigs in contaminated food pass to the caecum and colon and there undergo further stages of development leading to the production of adult worms. This course of life history had also been determined by Dr Goodey and an account of it published in the *Journal of Helminthology* in 1924.

With regard to *Metastrongylus apri* parasitic in the bronchi and bronchioles, where it gives rise to "husk," Dr Goodey explained that the eggs hatch in the lungs, larvae are coughed up and are swallowed. They pass through the gut unchanged and are found as free larvae in the droppings. Nothing further is known about their life history, for no other stages of growth are definitely known. It is not known whether these larvae are directly infective, but the view was expressed that they are probably not and that some course of development, possibly in an intermediate host, is required before the larvae reach an infective condition.

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SOME ASPECTS OF THE MORBID ANATOMY OF PLANTS

By E. J. BUTLER, C.I.E., D.Sc., F.R.S.
(*Director, Imperial Bureau of Mycology, Kew.*)

(With Plates VIII–XII and 13 Text-figures.)

ADDRESS OF THE RETIRING PRESIDENT OF THE ASSOCIATION OF ECONOMIC BIOLOGISTS, DELIVERED ON JANUARY 24TH, 1930.

REGENERATION.

THE work of Němec (24), Vöchting (43) and others has shown how completely the tissues of growing parts of plants, destroyed by traumatic action, can be, in numerous cases, regenerated from the most diverse sources. Not only can the conventionally distinct derivatives of the dermatogen and periblem be reconstituted from those of the plerome, and a cortex cell (if it be not too old) directly become epidermal by thickening its outer wall, acquiring a cuticle, and even growing out into a root hair, but the entire lost halves of a young tuber, stem, or petiole may be regenerated under suitable conditions in form and structure scarcely distinguishable from the normal.

Vöchting has also shown that a perforating wound may become surrounded, not only by a cork, but by a deeply seated cambium which may cut off phloem towards the wound and xylem deeper in, the part between the phloem and the cork coming to resemble a cortex.

That this process may be carried so far as the development of an epidermis lining the wound cavity would appear to be established by some experiments described by E. F. Smith (41). He inoculated young flower heads of sunflower with the crown gall organism, *Bacterium tumefaciens*, by needle stabs, and others were similarly treated but with a sterile needle. The result in both cases was the development of long cavities in the pith of the rapidly elongating stalk. That shown in Plate VIII, fig. 1, was 16 inches below the inoculated apex when cut, this distance representing elongation after inoculation. It shows the cavity surrounded by a more or less complete vascular ring with xylem outside, then a cambium cutting off phloem on the wound side, then bundles of fibres of the hard bast type, next a thin-walled parenchyma

resembling cortex, and finally, lining the cavity, an epidermis sometimes smooth, sometimes with hairs like those on the surface of the plant (Plate VIII, fig. 2). Quite similar structures, even to the epidermal hairs, were got when the sterile needle was used. Smith makes a suggestion that these structures may possibly result from carrying in, on the point of the needle, invaginated fragments of the meristem of the torus which become grafted or transplanted, inside out, in the pith and subsequently elongate with the growth of the plant. They are, however, more easily explained as regeneration phenomena like Vöchting's. Even naturally formed hollows in the stem of *Brassica oleracea* were found by Beijerinck (2) to become surrounded by a cambium cutting off phloem towards the wound and xylem on the outer side, and Smith (39) artificially induced a similar condition by injecting monobasic ammonium phosphate into the hollow of young internodes of *Ricinus*.

WOUND REPAIR.

The commonest form of replacement of lost tissues is by the process of callus formation, such as takes place in the healing of wounds under moist conditions.

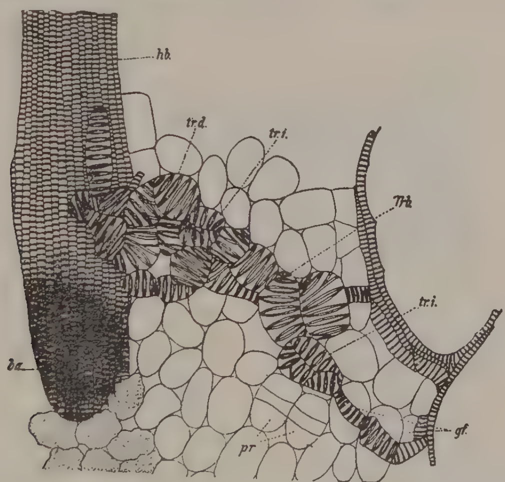
Callus may be formed from any living cell whatever under appro-

Text-fig. 1. Tracheid formation in the callus from a root of *Beta*. (After Küster.)

priate conditions, and the subsequent changes undergone in the callus mass are independent of the origin of the tissue.

The living cells bounding the wound that have not been killed in wounding grow out and form a tissue of dividing thin-walled parenchyma. In the deeper part of this the first differentiation frequently takes place

by the direct transformation of a callus cell, derived from any tissue, by reticulate thickening and lignification of its walls into a tracheid-like element (Text-fig. 1). This is one of the key processes in morbid anatomy. Several of these tracheids often develop close together, so that a little island of woody tissue results. Neighbouring groups may unite to form irregular tracheidal strands, varying in length and often broken; or an isolated group may become surrounded by a meristem developing in the cells bounding it on one or all sides, and this forms tracheids and wood fibres on its inner side. A procambial strand, forming wood and phloem, may appear at a somewhat later stage in the cells bordering



Text-fig. 2. Replacement of vascular connection destroyed by wounding in a leaf of *Impatiens*. Tracheids are formed directly from mesophyll cells (*tr.d.*) or after division of latter (*tr.i.*). A procambial strand is beginning to form at *pr*. (From Küster, after Freundlich.)

the tracheal strands, and may join up with vascular outgrowths from the original conducting system of the organ. Text-fig. 2 shows how readily resting cells of a tissue can, even without forming callus, restore a broken conducting tract by the direct or indirect transformation of the ground tissue into tracheids, outside which a new procambial strand may form. Sometimes, however, meristems develop in a callus not in association with tracheids.

Similar processes are extremely common in woody galls, the example shown in Text-fig. 3 being an island of tracheids in raspberry crown gall from a figure by Wulff (47). Phloem is not mentioned by him in these islands, but phloem strands are frequently found in the similar galls on

Rubus occidentalis that I have examined. The vascular system that supplies the adventitious cotyledonary leaves that are formed, as described by A. W. Hill (11), when the swollen hypocotyl tuber of *Cyclamen* is decapitated, arises, as was shown by Boodle (4), by division of one or more of the normal cortical cells (Text-fig. 3). There may be a central mass of xylem surrounded by phloem, or xylem and phloem pursue a wavy course, often rather widely separated from one another, towards the vascular ring. The elements in all these cases are very short, being limited by the diameter of the parenchyma cells from which they are formed, and the similarity of both processes is obvious.

The outer free zone of a callus may become corky by the development of a cork cambium, or may be a loosely united mass of cells re-

Text-fig. 3. On left, tracheid island in raspberry crown gall, with indications of meristematic activity in the surrounding gall parenchyma. (After Wulff.) On right, portion of trace of adventitious leaf of *Cyclamen*, cut longitudinally. *ph*, phloem. (After Boodle.)

sembling what is termed hyperhydric tissue. This arises from a meristem which cuts off roundish, loosely attached cells, resembling lenticel tissue, outside and thin-walled, callus-like cells inside. Similar tissue may form at the free surface without the intervention of a meristem. Very frequently also a large transverse cambium develops below the free surface of the callus and cuts off wood on its inner side and, less often, phloem and secondary cortical tissue on its outer side. Occasionally a layer resembling an epidermis clothes the surface. This is more frequently developed when a petiole or stem is split longitudinally, the callus outgrowth then, in favourable conditions, reconstituting the central cylinder by a cambium joining up with that of the uninjured half and forming not only phloem but a more or less normal cortex and epidermis outside.

The final stage of differentiation in a callus may be the exogenous formation of shoots from a mother cell or cell group at the surface of the callus or a few layers in from the surface. Even a callus derived from pith cells can give rise to these shoots. Their formation seems to be stimulated by excessive nutrition, for in uncongenial grafts (such as those between different species or genera of Solanaceae (16)) the upper side, which gets an excessive supply of nutriment from the scion, may form a callus very rich in shoots. Roots are often formed from callus, but, unlike the shoots, come always from the deeper layers. The various types of leafy outgrowths found at times on certain galls, such as the so-called embryomata of crown gall, are no doubt to be likened to callus shoots and need not be taken to imply (as has been suggested) that certain specific cells or cell groups preserve throughout their development a distinctive embryonic character, capable of regenerating the whole shoot system. In the processes leading to the formation and differentiation of callus, evidence can be found, in support of that given by other regenerative processes, that all living cells of the plant body have, at least, the potentiality of forming all parts of the plant. One of the effects of parasitism is, in some cases, to call out this potentiality, though usually in an irregular or unrhythmic fashion.

In callus proliferations the newly formed cells retain the power of division for, theoretically, indefinite periods, so that cells far removed from the wound surface continue to divide and form ever-increasing masses of thin-walled parenchyma. A second type of wound healing, however, is common in which the process resembles the normal growth in thickness by being the result of the division of cambial cells, the products of which, as usual, soon lose the aptitude to divide, becoming differentiated. The tissues thus formed are sometimes termed wound wood and wound cortex.

The largest masses of wound wood are formed from the original cambium stimulated into activity by injury to the bounding cells from parasitic or other causes such as frost. Cankers are due in large part to an excessive development of this tissue. In the case shown in Plate VIII, fig. 3, a tea canker which will be further considered below, the cambium was killed in places and repair by callus from the sides has occurred. Elsewhere it has only been stimulated, and wound wood has formed in direct continuation of the normal wood. Quite similar wound wood may be developed from cambiums formed in any part of a callus mass as already described. In this case only xylem may be formed, without phloem. Sometimes xylem is cut off on the outer side

of the cambium, sometimes on the inner, when the meristem develops, as is not unusual, around a tracheid group or a mass of sclerenchyma or a localised injury. In the first case there may be a small phloem group in the centre of the nodule, in the second either phloem tissue or parenchyma develops outside the cambium. Knobby isolated masses of wound wood thus formed are found in the secondary cortex of the apple and other trees and are well known in association with the phloem disease of *Hevea* rubber termed brown bast(5). Very frequently the wound wood is developed in vortex-like masses of fibres and vessels, forming knots of very characteristic appearance, probably as a result of pressure from unevenly developing meristems or uneven growth at the junction of normal and wound wood. Structures of the same kind are also found in wound bast and even, occasionally, in the tracheid groups that develop in leaf calluses. They are extremely common in woody galls, and beautiful examples can be seen in the woolly aphis gall and the olive knot disease.

Histologically, wound wood differs from normal wood in having less regular rows, composed of shorter elements, in the usually greater proportion of wood parenchyma, which may be found in species in which the normal wood contains none, in fewer fibres or even their complete absence, in thinner vessels, and in larger medullary rays. Its less solid construction renders it liable to repeated damage. Thus the new wood in frost cankers is readily again injured by frost.

Wound cork.

The result of wounding may be the production neither of callus nor of wound wood but of wound cork. This is a common result in all organs of the plant, especially in relatively dry air. The case shown in Plate VIII, figs. 4 and 5, is from preparations by my colleague, Mr Wiltshire, showing a relatively early and a later stage of wound inoculations of apple twigs with *Nectria galligena*. The cork tends to form parallel to the surface of the wound and often differs from normal cork in having thinner walls and larger cells. Every living tissue from epidermis to cambium or pith may join in forming this cork layer, which always tends to unite at its margin with the surface covering of the sound part, epidermis or normal cork as the case may be.

In leaves its formation is sometimes accompanied by a certain amount of swelling due both to hypertrophy (generally taking the shape of an elongation of the cells towards the wound) and to hyperplasy, as the result of the formation of walls parallel to the surface of the latter.

In citrus scab, microtomed material of which has been kindly lent by Dr H. S. Cunningham, Plant Pathologist, Bermuda, the organism (which has received several names—*Cladosporium citri*, *Sporotrichum citri*, *Sphaceloma fawcettii*, *Sphacelia citri*) causes a necrosis of a few of the superficial cells of the leaf (Plate VIII, fig. 6) and provokes the active division of the spongy parenchyma (8). The new walls are roughly parallel, in the early stages, to the necrosed surface and the process extends to a considerable depth, sometimes leading, in attack on the under surface, to divisions in the palisade cells on the opposite side. Near the lesion the cells elongate more towards the injured part and become more divided than those farther away, and in the layer of long cells with several cross

Text-fig. 4. Edge of cavity in leaf of holly mined by *Phytomyza aquifolii*, showing elongation of the mesophyll cells and their division by cross walls to form a phellogen. Intercellular spaces absent. (After Kerling.)

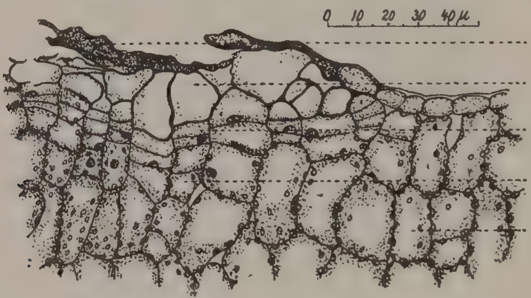
walls a cork cambium eventually develops (Plate VIII, fig. 7). This extends up to the epidermis all around the lesion and cuts off cork on its outer side and a little phelloderm on the side towards the sound tissues. All the primary cell walls of the hyperplastic area are thickened and intercellular spaces are much reduced.

Similar processes can result from insect injuries. Text-fig. 4 shows the margin of a larval passage of *Phytomyza aquifolii* (15) in a holly leaf, after the first year and after the insect has emerged. The passage has become more or less filled with large thin-walled bladder cells, and deeper in the mesophyll cells have elongated and formed a number of cross walls (up to 12), of which those towards the middle are close together and function as a phellogen, the cells on the wound side

becoming thin-walled cork, and those towards the healthy tissues phelloderm with thick, pitted walls.

Other types of injury can produce much the same effects. When savoy cabbage is exposed to tar vapour the leaves become spotted (15). The epidermis over the spots is killed (Text-fig. 5), while the first row of palisade cells elongates and may also broaden. The enlarged cells divide by three to five walls parallel to the necrotic surface, the outermost cells (those next below the epidermis) being very large, thin-walled, and suberised. The narrowest cells are about the middle of the original palisade cells and form a wavy line under the necrosed area, the cells on the outer side of this phellogen-like layer being corky and those on the inner side more normal and representing a phelloderm.

In most of these cases it is noticeable that the cork does not form directly under the wound, but is separated from the latter by one or



Text-fig. 5. Savoy cabbage leaf spotted from the action of tar vapour. Epidermis killed and a phellogen forming in the palisade cells. (After Kerling.)

more layers of large cells, sometimes bladder-like, and with walls and intercellular spaces impregnated with a substance resembling lignin but possibly a kind of wound gum. It is the cells below this zone that elongate and divide transversely, the middle divisions corresponding to a phellogen, with corky cells in rows outside and phelloderm rows below. There is usually a zone below the phelloderm in which the cells are enlarged, but little, if at all, divided. All this part is without intercellular spaces, and there is often no distinction between palisade and spongy parenchyma in it.

The metacutinised layers between the necrosed area and the cork correspond to the "blocking layer" of Priestley (30), and the whole process seems to agree closely with his description of the formation of wound cork.

All the living cells of the leaf may take part in these processes,

collenchyma and sometimes sclerenchyma cells (when young) apparently first losing their thickenings and then enlarging and even dividing.

When an organism penetrates the whole thickness of the leaf, the reaction is naturally restricted to the edges of the spot; the wound cork forms from the epidermis of one surface to that of the other, and the whole of the contained tissue may be cut off and fall out, producing the well-known shot-hole effect.

The cases cited show that there is nothing specific in the action of the stimulus. The reactions are similar to those found in the normal processes of the plant, *e.g.* below lenticels; they are latent in the plant; and the stimulus—fungus, insect, or injurious vapour—merely calls them into play. Even when the tissues from which it is derived are diverse, the tissue of reaction tends to be uniform. In the zone around the spots of *Clasterosporium carpophilum* on cherry leaves, for instance, not only is the differentiation between palisade and spongy parenchyma lost as a result of cell division, but the epidermal cells divide into cells very like the rest.

Lastly there are cases where no reaction follows the wounding, other than the deposit of suberin or lignin or wound gum in the walls of cells bounding the wound. There is no outgrowth of callus, no meristem to form wound wood or wound cork, and there may be no dividing walls formed in any of the cells near the wound. This type of healing is common in superficial wounds in roots and submerged aquatic stems, and Priestley (29) has correlated it with the presence of a well-developed endodermis, so that there is a barrier to the passage from the vessels of the solutes that are necessary for meristem formation.

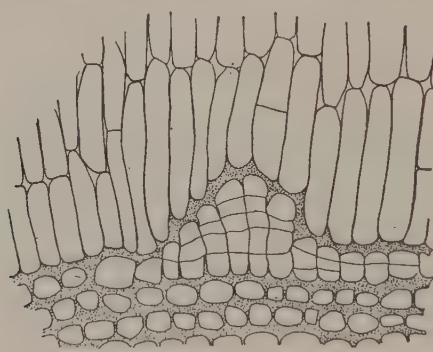
All sorts of intermediate conditions between these various reactions to wounding or necrosis may be found. Wound cork may form in part of a cut potato tuber and callus in another part. In one and the same cherry leaf spot caused by *Clasterosporium carpophilum* a good wound cork may be found in one place, while in another only the enlarged callus-like cells, becoming metacutinised, are formed. When an epidermis in some leaves is destroyed locally it may be replaced by a new epidermis with hairs and stomata derived from the mesophyll, or a kind of false epidermis merely with a cuticle but without hairs or stomata may form. This also occurs quite normally in citrus leaves, in which during development the upper epidermis often splits and is replaced from the mesophyll. There may be a very few cell divisions before cuticularisation. Here, as in many other cases, there is no sharp distinction between the regenerative and the callus processes of healing.

In general, every deep, closed wound or patch of necrosed tissue, even in secondary or so-called resting tissues, may become surrounded by a meristem, which may be directly formed from pre-existing cells, as in cork-cambium formation round the cavity, or may be preceded by cell proliferation to form a callus.

Anatomical changes caused by factors other than wounding.

All these processes of regeneration and repair result from wounding or injury. There is another great category of modifications in cell or tissue growth and differentiation arising from inner processes in which no gross injury is involved.

It is well known, for instance, that the mere placing of an actively growing shoot of many plants in unusually moist air causes the develop-



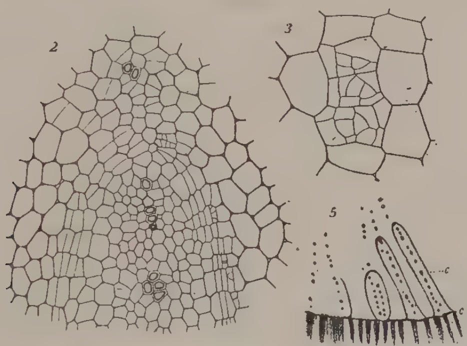
Text-fig. 6. Hypertrophy and hyperplasy of collenchyma of *Clerodendron* twig after vaselining. (From Küster after Schilling.)

ment of cellular outgrowths, intumescences, lenticular protrusions, and the like. These outgrowths are usually composed of rows of radially elongated, thin-walled parenchyma. In green stems and leaves the cells concerned are chiefly those of the epidermis, primary cortex, and mesophyll. These outgrowths can be produced even more readily if transpiration is checked by coating the parts with vaseline or paraffin. Text-fig. 6 shows how collenchyma (in the cortex of a *Clerodendron* twig) elongates and forms a thin-walled tissue after vaselining.

Not only humidity, but nutrition, light, and many other factors can cause deep-seated tissue changes. For such changes the terminology used by Küster (18) is convenient. When the result is under-development it is termed *hypoplasy*; when there is, on the contrary, a swelling, this may be due to a simple *hypertrophy* of the cells, that is to say, an increase

in size without division, or to a *hyperplasy*, which implies an actual increase in number of the cells. Sun and shade leaves, submerged or aerial leaves, etiolated shoots, starved or over-nourished plants all yield familiar examples of these anatomical modifications, due to what may be considered purely inner causes. How extensive such modifications may be was shown by some of Vöchting's (43) experiments.

When the kohlrabi plant is prevented from flowering in the second year by removing the flower buds as they form, the stem above the tuber swells, the increase being very strongly marked in the leaf base cushions. The cortex and conducting tissues become much enlarged. The collenchyma divides and forms a thin-walled tissue. The phloem is radially



Text-fig. 7. Swollen leaf base cushion of kohlrabi. Lower right-hand figure, diagram of new cambium formations in the enlarged medullary rays, tending to encircle one or two rows of vessels, but leaving the innermost outside the ring. Left-hand figure, detail of inner part of a row of vessels with lateral cambiums uniting in the protoxylem region and a few phloem elements forming on the outer side. Cell division active around the innermost protoxylem vessels. Top right-hand figure, a phloem nest in the vascular region of a primary bundle. (After Vöchting.)

elongated but not otherwise much altered. The xylem of the main leaf trace may become split into a number of bundles, each more or less completely surrounded by its own cambium ring (Text-fig. 7). This is due to a new formation of cambium on each side of the enlarged medullary ray, the cells of which first elongate tangentially before dividing and the process either extending in from the original cambium of the leaf trace or beginning in the deeper parts and extending outwards. The ends of these lateral cambiums may unite, as the result of the radial elongation and division of a layer of wood parenchyma, in the primary xylem, which may be so situated as to isolate from the rest of the bundle some of the primary vessels on the inside. The new cambium forms

phloem outside and xylem inside. Sometimes the middle part of the protoxylem parenchyma may become very active and form a thin-walled tissue in which nests of phloem develop, which may then become surrounded by a cambium, on the outside of which an occasional xylem vessel may be formed. These small inverse concentric bundles are also formed in the broadened medullary rays, and the smallest ones may have no cambium or xylem.

When side shoots take part in a similarly induced thickening the swelling may be due largely to increased cambial activity, but instead of normal wood a thin-walled tissue is formed (Text-fig. 8), inside which some of the primary vessels, with thicker-walled tissue around them, may be seen. The phloem is much increased in this case also.

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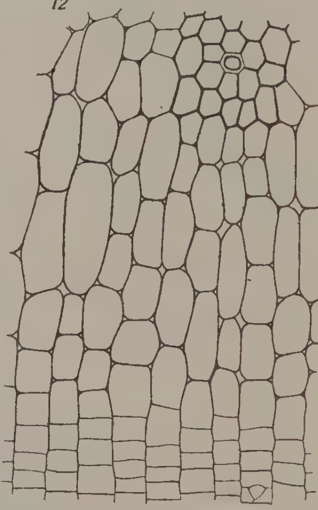


Text-fig. 8. Thin-walled parenchyma formed on inner side of cambium in swollen axillary shoot of kohlrabi prevented from flowering. A primary vessel surrounded by thick-walled cells is seen near the top. (After Vöchting.)

In similarly treated savoy cabbage Vöchting found concentric inverse bundles in the thickened roots and leaf base cushions, situated in the collenchymatous tissue of the inner xylem, though no bundles of this type are known in the normal plant as they are in normal kohlrabi. In the stem thickenings thus produced when the savoy is prevented from flowering, the phloem is much enlarged and, both in this plant and the kohlrabi, there is a considerable development of stone cells and of peculiar elongated branching sclerotic elements, termed idioblasts by Vöchting. These are found in the same region in some of the fungus galls to be referred to later. On the other hand, true fibres are noticeably scarce in the wood and phloem of the abnormal tissues in both cases.

One other experiment of Vöchting's requires mention. He succeeded in grafting young kohlrabi leaves into the medulla of a tuber, where they grew into abnormally large and thick organs. In the thickened petiole he found the bundles tended to become concentric.

Hans Winkler, a year earlier (45), had obtained a still more striking case of this formation of concentric bundles in the petiole. He was able to get well-developed flowering shoots from the leaves of *Torenia asiatica* cut off with the petiole and planted so that the latter rooted. The petiole

thickened when it thus came to function as a stem, and its normally flattened collateral leaf trace bundle became a round, closed woody cylinder (Plate IX, fig. 8). The change took place by the development of cambial divisions (Plate IX, fig. 9), at first in the old cambium which had become inactive and then in cells of the ground parenchyma, either extending out from the side of the old cambium and curving round till the two sides united, or in groups of isolated parenchyma cells opposite the central part of the leaf trace, the process spreading laterally and curving down to join the original bundle cambium. In the case shown, the former method has occurred and only the two cells in the middle above have not yet fully joined in the process of division. Some of the products of division have become rows of tracheids, and phloem elements are appearing in places. Ultimately there forms a compact woody cylinder at least twenty times the area in transverse section of the normal leaf trace. A small-celled phloem surrounds the wood, and all the elements formed outside the original bundle are short as they are limited by the diameter of the ground tissue cells.

Enough has been demonstrated in numerous investigations to establish the fact that, by purely internal processes and without the intervention of any parasite or agent of necrosis, every living tissue can become hypertrophied and all, by cell division, can show hyperplasy. In the hyperplastic tissues differentiation to other tissue forms can follow. Naturally, cells that have not finished growth and differentiation react the more readily, but even old cells can be affected, either directly or after first dividing and thereby becoming, so to speak, rejuvenated. Even cells that have developed thickened walls, as collenchyma, can enlarge and divide, or those in the early stages of lignification can be delignified, resume the parenchymatous condition, and behave like any other parenchyma. Differentiation in hyperplastic tissues may come from direct alteration of a cell—perhaps only one or two of a group formed from a single mother-cell—to a thick-walled type such as a sclereid, or to form a little island of phloem or a tracheid; or it may be indirect as the result of the development of a meristem in the new tissue.

So also tissues, cells, cell walls, and cell contents can show hypoplasy, or arrest of development or of differentiation, from internal, more or less natural, causes such as unsuitable nutrition or a deficiency in light.

Comparison between gall formation and other anatomical modifications.

The processes that take place in gall formation, especially those due to vegetable parasites, resemble those described above, allowance being made for the fact that the stimulus may be more localised and may act for a longer time than in the case of, say, a wound. It seems very doubtful whether there is any essential difference between the tissue changes in gall formation and those in restitution and repair, or when tissues are stimulated or under-developed as a result of internal adjustments to alterations in the food supply or the environment.

That it may not be superfluous to reaffirm this point of view is apparent from a cursory survey of some recent phytopathological literature. Statements can easily be found that all tumours arise from cambial activity, or at most from certain embryonic cambial derivatives that have conserved their meristematic characters; that leafy shoots can only come from certain totipotent cells that have preserved throughout their developmental history the power of reconstituting the plant; that vascular tissue, when it appears in a gall at a distance from the main conductive system, comes from special groups of cambium derivatives that would normally have formed vessels but are deflected by some agency into forming parenchyma without, however, losing their vessel-forming character; that concentric vascular nodules or cylinders in the pith and cortex come from complex invaginations or evaginations of the cambium; and that the presence of such structures in a petiole or leaf implies an intrusive growth of stem-forming tissues into the leaf. It is obvious that some of these statements cannot possibly be correct. No theory of persistent initials will serve to explain the large transverse cambium often formed from all the cells in a layer near the surface of a callus, derived as they often are from the most varied tissues. Nor will such theories explain the regeneration of the *Cyclamen* cotyledons nor the production of leafy shoots on callus from all kinds of tissue.

Crown gall.

Many of these views have been based on the phenomena observed in crown gall caused by *Bacterium tumefaciens*. No other plant gall has been so completely studied, not only on account of its intrinsic interest but also because of its suggested analogy to cancerous growths in animals. In none can the anatomical changes be better followed on

account of the magnificent series of photomicrographs with descriptive text in the numerous publications of Erwin F. Smith.

When growing stems of sunflower, tobacco, tomato, castor, etc., are inoculated with the crown gall organism by means of needle pricks, it is not uncommon subsequently to find closed vascular rings, spheres, or cylinders in the pith and cortex (42). When found in the pith the orientation of the tissues is frequently inverted, the phloem being inside and a ring of xylem outside the cambium. In the cortex and petiole (Plate IX, fig. 10) the orientation is normal and, as already mentioned, Smith interpreted these structures when found in leaves as evidence of an actual infiltration of cells bearing stem characters by a process similar to the infiltration or metastatic processes in animal cancers. The examination of his photomicrographs of the earlier stages of this formation, however, shows that it comes about as a result of division of the ground cells of the petiole outside the leaf trace. In one of these (Plate IX, fig. 11) the new cambium has as yet only joined up with the original cambium on one side. In another, at a later stage, the gap has been almost entirely closed (Plate IX, fig. 12), but there are still some ground tissue cells in the early stages of division. The centre of the new formation is a mass of tumour cells, but it is sometimes possible to make out that cells of the ground parenchyma are included within the ring. Robinson and Walkden pointed out in 1923 (36) the resemblance of this process to that described by Winkler in *Torenia*, and showed (what is already evident from Smith's photographs) that it resulted from the division of ground tissue cells opposite to the protoxylem, the latter being the region that they proved was occupied by the bacteria. Riker also (35) independently observed, in the same year, a tumour strand in the outer cortex of inoculated sweet-pea giving rise to a secondary gall with a closed vascular sphere (Plate IX, fig. 13). Serial sections showed that there was no connection between this vascular nodule and the vascular cylinder of the stem. Like Robinson and Walkden, he found (and this has since been confirmed by J. B. Hill (12)) that tumour-strand formation in this position and in the pith is due to extension of the bacteria along intercellular spaces running longitudinally in the stem (Plate IX, fig. 14) or drawn out by rapid elongation of young parts, the cells bordering the infected spaces being stimulated to rapid division. In the pith strands formed in the sunflower Riker found some showing only hypertrophy of the cells round the first-formed tumour cells, but in others the vascular ring was developing, with xylem outside.

Magrou in 1928 (21) endeavoured to explain the closed vascular rings

that he also observed in his crown gall inoculations by a hypothesis that is more ingenious than convincing. He supposes that the cambium is stimulated to tangential hyperplasy and, having no room to expand laterally, becomes puckered into folds, some of which bend into the pith and others out into the cortex, in both cases tending ultimately to become cut off from the parent cambium. This would explain the inverse orientation in the pith and normal arrangement in the cortex that has usually been observed. Or he supposes that in needle-prick inoculations the ruptured cambium grows out and in along the edges of the wound, uniting at its base in the pith but recurving in the cortex where the wound prevents union. This explanation altogether fails to account for the concentric leaf trace bundles shown above, or for the absence of any remnants of a vascular connection with the main conductive system, or for the fact that the centre of these structures is usually occupied by a core of tumour cells, often in longitudinal connection, above and below, with a non-vascularised strand of such cells extending from the point of inoculation. Still less would it account for the similar structures shown by Smith in the wood ring itself, situated in the protoxylem region, where he has also figured a little nest of phloem in an inoculated sunflower. It will be remembered that Vöchting obtained similarly situated phloem nests in his kohlrabi plants prevented from flowering, and found that they represented an arrested early stage of inverse bundle formation.

There need be no doubt that Robinson and Walkden's and Riker's explanation of these formations as due to the presence of bacterial infection in elongated intercellular spaces or protoxylem vessels, acting as a stimulus which causes hyperplasy of surrounding cells, is correct, and that they are not associated with any activity of the normal cambium but are at a distance from, and quite independent of it.

The inverse arrangement of the bundles of the pith, when such are found in crown gall, need cause no surprise. Medullary bundles are not uncommon in many families of plants and are frequently concentrically inverse or sometimes composed of phloem only (13, 22). Various explanations, not excluding one on which Magrou's theories are based, have been offered of their inverse arrangement, none of which appears very convincing.

Normally orientated cortical bundles or nodules, isolated from the main conducting system of the stem, occur in at least two other woody galls due to bacteria. In the olive knot caused by *Bacterium savastanoi* I have found beautiful examples, and they occur also in the bacterial

tumours of the Aleppo pine described by Prillieux (31), Petri (27) and Dufrénoy (9), the latter author, who has figured one, taking it to represent the core of an adventitious bud which had failed to emerge from the cortex and remained permanently buried. Petri, however, showed that they were formed by a meristem developing around bacterial foci, as in crown gall.

Bacterial infections are perhaps more likely to cause tissue changes localised around a focus than are other parasitic attacks, as the organisms are commonly confined to a restricted space, such as a single elongated intercellular space. It is easy to find in even fairly old crown galls appearances such as that in Plate IX, fig. 14, where extension of the parasite along intercellular spaces in the subepidermal layers of a tomato stem at some distance from the point of inoculation has led to two small areas of commencing hyperplasy of the type that leads to the formation of "tumour strands." I have found quite similar structures in tomato galls 3 months old, kindly communicated by Mr R. V. Harris.

Though at first Smith believed these "tumour strands" to be actual intrusive growths of the tumour cells insinuating themselves between the pre-existing tissues, he later recognised that some at least resulted from the division of previously normal cells along the path of the strand and stated that "Possibly all do so" (40).

In the areas of rapid gall formation it is easy to follow the conversion of normal cortex cells into gall tissue. In Plate X, fig. 15, a tangential section has been cut of a tobacco stem gall. At *E.* the epidermis can be seen, at *Cr.* crushed cells in the outer cortex. Riker has shown (34) that the cells thus formed in tomato continue to be smaller and smaller by division up to over a month old, then enlarge somewhat. The average size of the normal cortex cell is nearly 5000 sq. μ, and this fell to 166 after 33 days, then rose again to 407 sq. μ in 58 days.

The tumour of the type shown breaks through the vascular cylinder by the path of the medullary rays. Fig. 16 is from a tangential section of the same tumour and shows the gall cells formed in a ray. During this process extension up and down—mainly up—may take place in the form of narrow strands, marked here and there by little masses of gall tissue. These strands are usually in the protoxylem in *Chrysanthemum frutescens*, a plant in which the apparent upward extension is due, as Robinson and Walkden have shown, to elongation of the infected part.

The crown galls hitherto mentioned have been in herbaceous stems, and in the early stages these are marked by localised areas of reaction in the cortex, vascular cylinder, or pith. Another type of considerable

interest is found in the more or less woody stem or cane of the raspberry. In a fruiting cane, that is a cane in the second year of growth, the galls appear on the stem of the American black raspberry, *Rubus occidentalis* (specimens of which were kindly communicated by Dr Riker), as multiple outgrowths on the above ground parts. A transverse section of one of these in the early stage (Plate X, fig. 17) shows that the hypertrophy is at first confined to the region of the pericycle outside the fibre bundles. There are no nest-like groups of tumour cells such as are found in the plants previously considered. The swelling consists of thin-walled radially elongated parenchyma in rows and with scanty contents. The multiplying cells lie below the cork and are at first external to the groups of pericyclic fibres. At this stage they may be considered to be a parenchymatous phelloderm, but the reaction soon extends down between the fibre bundles and involves the tissues between them and the outer part of the phloem. The growth in this position bends up the pericyclic fibres and separates them widely from one another. The hypertrophy and hyperplasy rapidly increase (Plate X, fig. 18), but there is little cambial stimulation. Here and there (usually not over the whole arc of cambium underlying the gall) there is a small outgrowth of gall wood, which is sharply differentiated from the normal xylem by the scarcity of fibres and the thinner walls and wider lumina of the vessels, which are often distorted in their course. Outside this a patch of active cambium is found, and over it the phloem. The margin of the tumour is still mainly pericyclic, but the central mass is of more doubtful origin, being probably partly pericycle, partly phloem and even possibly partly cambium. Out in the gall parenchyma there early appear single tracheids or tracheid groups, just as in an ordinary callus and, like these, extremely short. One of these groups from the gall on the allied *R. idaeus* is shown in Text-fig. 3 on p. 178. As in callus, too, the tracheid groups sometimes become bordered by a meristem, and develop further tracheids on one side and phloem or gall parenchyma on the other. The gall often becomes highly vascularised by this means and is penetrated in all directions by irregular vascular strands, some of which can be traced into the original vascular cylinder.

At the surface the cork is early ruptured, and though there may be some attempts at renewed cork formation, the surface of the gall is usually a loose mass of brown collapsed cells giving a weak lignified membrane reaction, and resembling the so-called hyperhydric tissue already mentioned as one of the tissues frequently formed on the surface of a callus.

Sections cut from galls resulting from inoculation with *Bacterium tumefaciens* on the ordinary red raspberry, *Rubus idaeus*, kindly provided by Mr R. V. Harris, resemble those on the other species, though the well-marked corky layers that form the so-called polyderm in the endodermal region make it even easier to determine the origin of the gall below this position.

OLIVE KNOT.

In the olive knot caused by *Bacterium savastanoi* (1, 31) the galls are also formed on woody stems. In the primary gall the part external to the cambium is mainly involved, the cells of the outer cortex, immediately below the periderm, forming an important part of the gall, which may, however, involve the inner cortex, the pericycle, and the phloem, all of which may show a marked hyperplasy around the bacterial pockets. The latter are more marked than in crown gall, as the organism causes necrosis of small masses of tissue so as to form obvious cavities. The swelling pushes out and ruptures the cork (a subepidermal one in the olive), and also separates the bundles of pericyclic fibres from one another. The process extends to the phloem parenchyma, so that the sieve tube groups and fibres are similarly dispersed. It becomes almost impossible to differentiate the various tissues external to the cambium from one another, phelloderm, cortex, pericycle, and phloem being composed mainly of oval gall parenchyma interspersed early with pitted sclerenchyma and tracheids. The xylem is not directly involved, but the cambium is stimulated into increased activity to a certain extent so that the woody cylinder becomes thicker than normal, while the medullary rays are increased in diameter. The bacteria reach the inner wood probably along the rays, and extension, mainly in an upward direction, occurs in or near the protoxylem, where the tissues seem to be particularly readily penetrated by pathogenic bacteria. This migration of the bacteria results in the formation of secondary tumours as in crown gall. In these secondary tumours Schiff-Giorgini (37) states that the bacteria are always found in the wood.

In the cortical gall tissue of olive knot, tracheids eventually appear and meristems may form which develop spherical or cylindrical nodules of wood surrounded by a cambium, which may cut off phloem or parenchyma on the outer side. When the strands are elongated, the usual twisted or vortex type of tracheid tissue may result, as in callus wood. By the time the galls have reached a fair size they are largely composed of lignified tissue, and the bacterial colonies are found in the

midst of nests of gall wood, around which is still a fair amount of gall parenchyma. The vascularised tissue of the gall unites with an outgrowth of traumatic wood from the vascular cylinder of the axis, which spreads out into the gall.

In the form on the oleander (38), caused by a variety of the same organism, the infection channels are stated to run chiefly in the cortex and the cells around the bacterial cavities show hypertrophy and hyperplasy, resulting in a sheath of small, thin-walled, cuboidal cells.

Other gall formations in tissues mainly external to the cambium.

Another type of gall in which there is no indication that the cambium is the tissue mainly concerned, due this time to an insect, is that caused on various conifers by *Dreyfusia nüsslini* so well described by Chrystal (6). Here again the stimulus is localised, even more so than in the bacterial galls, and probably, too, acts for a shorter period than in the latter. The attack (which may be multiple) takes place at an early stage in the bud or just below, in the former case sometimes before lignified xylem has been developed. It causes an arrest in the formation of xylem, and hypertrophy of the phloem and medullary rays, sometimes extending through the latter to the pith. The stylets can sometimes be traced half-way down a ray but usually end in the phloem; they are unable to penetrate the xylem after lignification sets in. In their immediate neighbourhood the cambium ceases activity. In early attacks, before xylem has been differentiated, the whole or a part of the procambial strand in the neighbourhood of the stylet becomes a gall tissue of large, densely filled cells with prominent nuclei. In attacks lower down the shoot, the cortex may also be involved.

Sometimes in multiple attacks the xylem is separated into isolated bundles, and when this occurs a cambium may form laterally along each bundle in the medullary rays and internally in the pith, so that the whole bundle becomes surrounded by cambium. The tendency for bundles isolated by enlargements of the medullary rays to become surrounded by cambium was already noted in Vöchting's experiments described above, and is one that is frequently observed in both insect and fungal galls.

In the gall formed by *Phylloxera vastatrix* on vine roots, attack on a young root may equally lead to an arrest of development, the xylem being replaced by parenchyma where the stylet has penetrated, to a greater or less depth—sometimes completely in very early attacks before

differentiation has taken place. The rest of the root cortex in this case hypertrophies and the cells divide freely, while the xylem has short and very broad vessels. Petri (28) has clearly shown, however, that in the *Phylloxera* gall the effect on the tissues depends on the age of the root attacked. In somewhat older roots, where differentiation has progressed further, the pericycle, which is the region of cork formation in vine roots, shows hyperplasy all round, and the cortex becomes swollen laterally. Still later the cambium reacts actively, cutting off parenchymatous xylem internally of the same type, as will be mentioned later in the woolly aphis gall and, like the latter, becoming more normal again as the stimulus passes off, while the now well-developed pericyclic phelloderm of the secondary cortex is also multiplied. In still later stages the reaction fails to reach the cambium, and the swelling is chiefly due to the parts external to the phloem.

These cases are enough to show how incorrect is Dufrénoy's statement (9) that tumefaction is essentially a cambial activity, or M. T. Cook's (7) that galls only result when the excitation or stimulant is applied to the meristematic tissue.

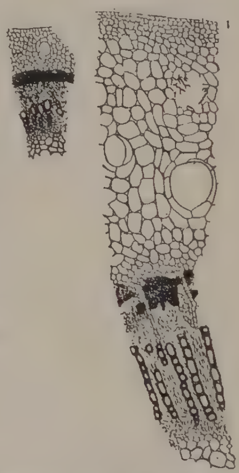
Galls involving cortex and vascular cylinder.

Witches' broom of cacao.

The next type of gall to be mentioned is one in which cambial activity plays a part more or less equal to that of other tissues. In the witches' broom of cacao, caused by *Marasmius perniciosus*, the stem is abruptly swollen to twice or three times the normal diameter. The swelling may involve everything from the epidermis to the centre of the stem. In a case measured by Went (44), the cortex, phloem, and xylem were each about three times their normal thickness, and the pith nearly twice as broad as lower down. The relative amount of thickening in the different tissues is, however, very variable. In the section shown in Plate X, fig. 19, the cortex is entirely crushed by the growth of the pericycle and deeper tissues. The intercellular hyphae of the parasite were found in the outer phloem near the point where the swelling commences, *i.e.* the oldest part of the gall. Here there is a slight proliferation radially of the one or two layers of pericycle just outside the outer fibrous layer and between the latter and one or two layers of inner cortex, rich in starch. Then there is a rapidly increasing proliferation of phloem parenchyma deeper in, which pushes apart the bundles of fibres in the phloem and separates them from one another. The crushed cortex

is still clothed by the remains of the piliferous epidermis. The brooms are short lived (some 6 weeks) and remain soft and fleshy, cork being rarely formed on them. In the cases described by Went, the mycelium at the base of the gall was confined to the cortex, and in his cases the cortex was not crushed but considerably thickened. Sometimes the xylem thickening may be about equal to phloem, pericycle, and cortex taken together. The medullary rays are broadened, especially in the outer part of the phloem, which is narrowed to a series of pointed wedges. It becomes almost impossible in this case to distinguish the cortical parenchyma from that deeper in, except that the pericycle can still be traced by its scattered groups of fibres. All the parenchyma, except that nearest the epidermis, comes to assume the same shape, elongated radially and with thin walls. This parenchyma multiplies by the division of all its cells, but mostly those of the inner cortex and outer phloem and, especially, the cells lying immediately next to the phloem fibres. The xylem vessels are broader and with thinner walls, and the outer xylem elements are largely parenchymatous. As one passes higher up the gall, the fungus is found throughout its thickness from the pith to the epidermis. The higher up one goes, too, the less clearly the different tissues are differentiated from one another. The fibres become at first septate, then reduced in numbers, and finally none forms.

Most of the thickening of the xylem seems to be from cambial activity, but in the other tissues it is mainly due to division of parenchyma *in situ*.



Text-fig. 9. Comparison of normal and swollen midribs of leaves from cacao, to show the effect of *Marasmius perniciosus*. (After Went.)

In the swollen leaves of this gall the distinction between palisade and spongy parenchyma disappears (as is common in leaf galls). In the midribs of sound and diseased leaves shown in Text-fig. 9, the cortex is over six times, the phloem nearly twice, and the xylem three times as thick in the latter as in the former. The xylem vessels are larger and more numerous, the medullary rays much wider and the pericyclic band of fibres broken up.

Acacia gall rust.

Another gall in which pericycle and phloem are extensively involved but cambial activity also occurs, is that of *Acacia decurrens* caused by the rust *Uromycladium notabile*, specimens of which have been kindly communicated by Dr G. H. Cunningham of New Zealand. In this case the relationship of the tissues is difficult to make out unless they are followed from the very early stages (Plate X, figs. 20 to 23). In the rachis of the bipinnate leaf there are two large bundle groups, each formed of a flattened band of xylem capped by a curved mass of phloem. The gall seems always to affect the bundle on the side opposite to that on which the leaflets are inserted in two rows near together. In the earliest stage (Plate X, fig. 20) there is a slight swelling in the middle of the phloem, where a group of cells becomes enlarged and thin-walled. This swelling extends, expanding the phloem into a conical mass (Plate X, fig. 21), the outer elements of which are large and thin-walled while those nearer the cambium are more normal. Soon the xylem is affected, and the new elements cut off from the cambium on that side show an excess of parenchyma with few wide vessels, though files of relatively thin-walled tracheids are still formed. There is an increase in the breadth of the rays and the formation of new ones and of much wood parenchyma. Fibres are still found scattered in the wood. The xylem hypertrophy, however, may not keep pace with that of the phloem and may come to an end fairly soon (Plate X, fig. 23). In the phloem there is a development of stone cells, and elongated fibre-like, thick-walled elements appear here and there, and seem to be similar to the previously mentioned idioblasts of Vöchting, which develop in the same region in Brassicas thickened as a result of the removal of the flower buds. The pericycle may also enlarge by the formation of radial rows of thin-walled cells, whose radial walls are in the same straight line. Groups of thin-walled dividing cells appear in the pericyclic fibre band and may divide the latter into segments. A cork may form in this zone, or the outer cells may proliferate as hyperhydric tissue. For a considerable time, however, the swelling may remain covered with the epidermis, bearing hairs.

In the early stages an irregular, sometimes discontinuous cambium can be made out between the xylem and phloem (Plate X, fig. 23), but the cambial cells usually lose their individuality later on and, with the outer part of the parenchymatous xylem, become involved in the general hyperplasy, so that it becomes impossible to differentiate between phloem, cambium, and outer xylem.

Differentiation of the type repeatedly referred to takes place by the direct change of some of the gall parenchyma cells into tracheids, or the development of isolated meristematic strands forming both xylem and phloem, which may be close together or diverge somewhat in their irregular course. Whorls and woody nodules are thus formed.

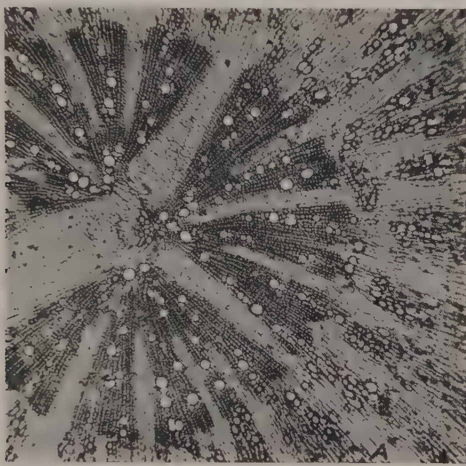
The hyphae in the early stage can be distinguished by their finger-shaped, sometimes branched haustoria in the enlarged phloem and, later, are easily made out in the pericycle and the vicinity of the cambium. Sections of the early stages have sometimes revealed them in the outer xylem, and in these cases the greater part of the young gall may be composed of modified wood. The galls, I am informed by Mr Rodda, Manager of the Te Kauwhata Horticultural Station, New Zealand, usually appear in late summer (February), and growth continues through the winter into November or December. The spore masses then appear. On small branches the galls usually die after the first crop of spores (insects often destroying them), but on large branches or stems they may go on growing for several years by renewed warty outgrowths, and ultimately reach a foot or more in diameter, being amongst the largest fungous galls known.

Galls involving mainly the vascular region.

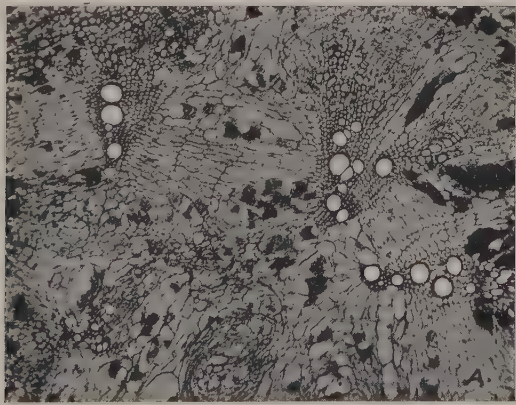
Club root.

There are a few galls that chiefly involve the vascular ring. In the club root of crucifers caused by *Plasmodiophora brassicae*, Kunkel (17) found that the swelling on cabbage stems caused by artificial inoculation at first affected the secondary cortex (the primary cortex having already been lost), but the organism reached the cambium fairly soon and produced a marked hyperplasy of the cells in this region. The elements cut off on the xylem side remained undifferentiated and again rapidly divided. In infections at later stages of growth, the medullary rays chiefly become infected and show a hypertrophy and hyperplasy which may be extremely marked (Text-fig. 10). Except at the point of original invasion, the phloem and secondary cortex only become infected outwards from the cambium. In stems which are no longer young, and in which the phloem tissues are hardened, only those cells that have developed after infection has reached the cambium become involved, if the medullary rays be excepted. The increase in size of the medullary rays may be such as to divide the vascular ring into widely separated

bundles. Distortion from this uncontrolled growth of the ray parenchyma may be such as to turn the bundles through 90 or even 180 degrees.



Text-fig. 10. Part of central cylinder of cabbage stem inoculated with *Plasmodiophora brassicae*, showing enlargement of medullary rays. (After Kunkel.)



Text-fig. 11. Later stage than Text-fig. 10, showing disorganisation of the wood by proliferation in the medullary rays. (After Kunkel.)

Not only is the original xylem broken up into separate masses (Text-fig. 11), but tracheidal tissue may develop in the hyperplastic ray parenchyma so that very confused structures result.

Woolly aphis.

In the tumours produced on the apple by the woolly aphis, *Eriosoma lanigerum* (32), the alteration is practically confined to the xylem ring of young shoots, the cortex, phloem, and pith being unaffected (Plate XI, fig. 24). In this case, it appears to be practically certain that the stimulus acts on the cambium mainly or exclusively. The first change is the suppression of the wood fibres, which are replaced by parenchyma; then the tracheids become thin-walled and are also largely replaced by parenchyma in rows of cells with pitted walls and sometimes containing starch. The newly formed wood is not lignified and mainly consists of wider cells than normal, with soft walls. Sometimes groups or layers of lignified wood are formed, especially in the later stages, so that patches of new xylem may appear at a distance from the old. Isolated cells or small groups in the deeper layers of the gall tissue may also become lignified. The medullary ray cells also hypertrophy and become thinner-walled than normal and, like the other parenchyma of the gall, divide in all directions so that their identity is lost. The sclerenchyma bundles of the pericycle become pushed apart by the radially elongated gall parenchyma, the unaltered cortex is split by the pressure, and the soft gall tissue protrudes. Cork forms on this, but in the winter cold the gall collapses and dies. Callus formation then ensues, new infestations occur on the callus masses and on the exposed gall tissue, and supplementary growths are produced.

It has been shown that the stylets reach the phloem, but do not penetrate further than to within three or four cell layers from the cambium, so that the stimulation of the latter acts at some distance. Why the action should be restricted to one side only, and that the side furthest from the stimulus, is not clear.

Tea canker.

Another case in which the gall is wholly woody is that known as tea canker, which has been attributed to various organisms, such as *Nectria* and *Macrophoma*, but the etiology of which is still obscure. The affected branches are swollen irregularly, the swelling usually surrounding the whole stem but being sometimes unilateral (Plate VIII, fig. 3), and the cortex is cracked and shows protrusions of reddish woody tissue. In the early stages of a thickening of the whole circumference, a transverse section (Plate XI, fig. 25) shows what appears rather like two annual rings of wood. Above and below the swelling, however,

these rings are not visible, but only a continuous body of xylem corresponding to the inner one. Hence the outer ring is a local formation, and as the other tissues are not appreciably swollen, the visible thickening is due to this excessive xylem development.

The new growth is due to cambial stimulation mainly on the xylem side, as in the woolly aphis gall. The gall wood elements are often not in radial continuation with the old, but are variously bent or contorted, sometimes even coming to lie across the rows. The pressure ruptures the cortex, and the bark becomes rapidly exfoliated into shreds. Sometimes the cambium is killed over a part of the circumference, and this leads to a complication of the structure by attempts at a normal process of wound repair extending in from the cambium at the sides (Plate VIII, fig. 3). The wound may be entirely closed, or repair may fail to close it and a gaping canker down to the original wood remains, bounded by swollen callus lips.

Another point of resemblance to the woolly aphis gall, and also to some of the *Phylloxera* galls, is that in many cases the more recently formed wood gradually comes to resemble the normal wood, with clearly differentiated vessels and fibres, as if the stimulus were passing off.

Sorosphaera veronicae.

Before leaving the consideration of galls involving the vascular system, a tumour that is usually confined to the procambial region behind the apical meristem may be referred to. This is the gall of *Veronica Chamaedrys* caused by *Sorosphaera veronicae* (3), one of the Plasmodiophorales and therefore in the same group as the club root organism. Specimens and microtomed sections of this have been kindly provided by Dr Schwartz.

In the longitudinal section shown (Plate XI, fig. 26) the tumour extends backwards on both sides from the point in the procambium where the xylem is just beginning to form, and also out into a leaf. Spiral vessels can be found throughout its length, but do not occupy any fixed position in the mass of gall tissue, being sometimes on its inside near the pith, sometimes on its outside towards the cortex and with only a few procambial elements between, sometimes in the middle. Evidently it is not only cells of the cambium, but also the derivatives of these lying around the protoxylem vessels, that become stimulated to increased growth and division. There is a great hyperplasy of the whole of the procambial region to form a tissue of more or less polygonal cells, in which are mingled the few xylem elements already differentiated

before the parasite reaches them and some scattered groups of elongated cells. Many of the infected cells and some of the neighbouring non-infected ones are also hypertrophied, sometimes greatly. As shown in Plate XI, fig. 27, there is no extension into the cortex or pith, but other sections have revealed a commencement of cortical invasion by an outward passage of the parasite from the vascular region through the endodermis. This invasion of the cortex from within, and the predilection of the parasite for the cambial region, are points of similarity to club root.

Galls involving primarily the pith.

Hypertrophies of the pith are commoner in insect than in fungous galls, as there is a large number of insects whose larvae inhabit the pith: many lepidopterous stem galls are of this type. The pith cells have remarkable powers of reaction by enlargement and division, and naturally when the woody cylinder is already formed in the stem the result of a pith swelling is that the bundles are forced apart and undergo various modifications.

In the insect galls due to larvae which occupy a pith cavity, the cavity is frequently surrounded by a nutrient tissue of densely filled cells formed by the division of the pith cells, and outside these is a zone of sclerenchyma, similarly derived, forming a protective layer. When the enlargement is considerable, these tissues are irrigated by vascular tissue joining up with the normal vessels of the stem. The stem bundles are forced apart by the enlargement of the medullary rays, and their number may be increased.

The example shown (Text-fig. 12) of the stem gall of *Potentilla reptans* caused by *Xestophanes potentillae*, Villers, from Houard's remarkable work on stem galls (14), indicates how several of these modifications come about. The cells around the gall cavity are elongated and much divided as a preliminary to the development of the nutrient tissue. Up to 30 or 40 cells may be formed by subsequent division in all directions in an original pith cell; in some other similar galls considerably greater numbers may develop. This hyperplasy extends into the medullary rays, and at the same time there is increased activity of the cambium, which forms secondary non-lignified xylem and parenchymatous secondary phloem. The cambial activity extends out into the greatly enlarged rays and down along the margins of the bundle, and may bend in at the perimedullary region so as to form a ring, or may extend towards the nutrient tissue of the gall. The irrigation of the latter is provided by

vessels developed from this cambium. The protective zone of sclerotic tissue appears between the new vascular tissue and the nutrient zone. In the formation of these protective layers a single mother-cell may contain parenchymatous daughter-cells in one part and sclerenchyma in another, a phenomenon frequently observed by Vöchting in his regeneration experiments.

Text-fig. 12. Part of vascular region and pith of *Potentilla reptans* stem inhabited by the larva of *Xestophanes potentillae*, showing cambial activity extending down into the pith. The pith and ray cells are elongated radially to the bundle and much divided. The insect cavity is beyond the top of the figure, and the primary phloem at the bottom. (After Houard.)

The similarity of the process of development of these lateral cambiums, and of the parenchyma in the protoxylem region, to what has already been seen in the thickening due to prevention of flowering in the kohlrabi is evident. As already mentioned in considering the *Dreyfusia* gall, there seems to be a general tendency, when the medullary rays exceed a certain size, for the bundles to surround themselves with a ring of cambium.

In some similar galls, *e.g.* that produced by *Aulax* (*Aulacidea*) *hieracii* on *Hieracium umbellatum*, Houard notes that the newly formed small bundles may consist solely of phloem in the early stages, and when xylem appears it may develop around a core of phloem to form an inverse bundle, again as in Vöchting's experiments.

Naturally the outer layers, pericycle, cortex, and epidermis, have to be modified to allow for the expansion of the stem that results from pith-inhabiting larvae. In some cases, *e.g. Epilobium montanum* stems swollen by the larvae of the lepidopterous *Mompa decorella*, an active pericyclic cambium develops, but instead of cutting off cork outside and phelloderm inside in the normal way, it forms thin-walled parenchyma on both sides. In this gall also there is an active formation of small cells in the hypertrophied endodermis, and also a very active division of the parenchyma cells of the internal phloem that is found in this plant. The cortical swelling may be due to simple hypertrophy, cells from a half to over one millimeter in length being found in the cortex in several of the galls described by Houard.

GALLS RESTRICTED TO THE TRUE CORTEX.

Purely cortical swellings, in which the central cylinder plays no part, are familiar in endotrophic mycorrhiza, where the endodermis of the infected root is not penetrated, and only cells of more or less defined cortical regions are hypertrophied. Some of the bacterial root nodules of the Leguminosae are also purely cortical formations, *e.g.* those of the French bean (23), though in others the central cylinder participates by a swelling corresponding in origin and position to a lateral root and joining up with the cortical swelling outside. In the common stem gall produced by *Protomyces macrosporus* on *Aegopodium podagrariae* there is a hyperplasy of the outer cortical cells restricted to those in the vicinity of the intercellular hyphae, the presence of which, in the early stages, can be postulated whenever a fully formed cortical cell is observed to be dividing. Though in this species of *Protomyces*, the vascular tissue is wholly unconcerned, in *P. pachydermus* on dandelion the spores occur in the phloem of the midrib and lateral bundles (Plate XI, fig. 28). This is only one of many cases in which two allied fungi produce different effects on the host, even when, as Magnus pointed out (19), both attack the same plant. Thus the aecidial stages of two species of *Puccinia*, *P. graminis* and *P. arrhenatheri*, both on the common barberry, produce, in the one case, localised leaf hypertrophies and in the other, witches' brooms, the differences being due, no doubt, to the widely different modes of infection in the two cases.

LEAF GALLS CAUSED BY FUNGI.

Leaves offer many excellent examples of the development, without the intervention of a meristem, of gall tissues composed of uniform cells, even when derived from cells that are normally quite distinct, as the palisade and spongy mesophyll. In the common abnormal growth caused by *Cystopus candidus* on the Shepherd's Purse (*Capsella bursa-pastoris*) the mesophyll of the thickened leaf is composed wholly of large round cells. Leaves of witches' brooms formed by species of *Taphrina* are also usually composed of round cells, with no differentiation into palisade and spongy layers and with small intercellular spaces. *T. deformans* also thickens the leaves, and causes the palisade cells to multiply and lose their normal shape, becoming almost isodiametric in the outer layer, where they are hard to distinguish from epidermal cells, and oval deeper in. *Exobasidium oxycocci* makes all the mesophyll into a uniform type of parenchyma without intercellular spaces in *Vaccinium macro-*

carpon and *Gaylussacia dumosa* (26). In *E. vaccinii* (46), on the other hand, the swollen leaves of *V. vitis idaea* preserve the palisade but little altered, the mass of the swelling being due to hypertrophy and hyperplasy of the spongy mesophyll, the cells of which are roundish or polygonal without intercellular spaces. In *E. rhododendri* on *Rubus ferrugineum* and *R. hirsutum* the effect is the same as in *E. oxycocci* when the leaf is totally infected from an early stage, but is like *E. vaccinii* when only a part of the leaf shows the swelling (10).

Text-fig. 13. Aecidial stage of *Gymnosporangium juniperi-virginianae* on apple leaf. (After Reed and Crabill.)

In the aecidial stage of *Gymnosporangium juniperi-virginianae* on the apple leaf (33), the hypertrophy is almost all due to the spongy mesophyll, the palisade being little altered (Text-fig. 13). The middle layers of the mesophyll are enormously elongated, and divided by transverse or oblique walls. It is in this tissue that the aecidia form, and its elongation gives room for the development of these organs in the deeper part of the leaf. Intercellular spaces are lost. This type of swelling resembles that sometimes found in leaves exposed to excessive moisture so as to cause the production of the intumescences already referred to.

Epidermal galls.

The epidermis may be directly involved in gall formation, as in the well-known host cells of *Synchytrium*, or may be indirectly concerned, as when it shares in the reaction of tissues around an invaded cell or other centre of irritation. In the latter case, it frequently tends to lose its characteristics as a distinct tissue. In the willow leaf galls caused by hymenoptera of the genus *Pontania*, Werner Magnus (20) found the epidermal cells dividing to form up to four daughter cells, and the underlying palisade up to eight, with a marked reduction in the differentiation between the two tissues.

In the capsule or sheath of small cells that forms around the single enlarged epidermal cell of the dandelion infected with *Synchytrium taraxaci*, microtomed material of which has been kindly provided by Dr W. R. Ivimey Cook, both epidermis and subepidermal cells are represented (Plate XII, fig. 29), though it is not possible to detect any difference in the daughter-cells of the two tissues. Here there is no cork formation, the small size of the source of the stimulus (localised to a single cell) and its relatively short duration, perhaps, being insufficient to induce any attempt to occlude the affected part. The cells elongate tangentially to the host cell and divide by walls at right angles to the latter. Intercellular spaces do not occur in this new tissue.

Localised stimuli that do not lead to cork formation are caused by several other epidermal parasites amongst the fungi. *Urophlyctis kriegeriana*, for instance, occupies a single epidermal cell, like *Synchytrium taraxaci*, but causes a somewhat more extensive reaction of the epidermis of the host (*Carum caruii*) with less penetration into the tissues below. On young potato stems the wart fungus, *Synchytrium endobioticum*, similarly stimulates the epidermal cells next to that in which the presorus develops to grow out into a rosette of club-shaped divided cells, and it is not until a later stage, when the resting form of the fungus has been carried deep into the tissues by repeated transverse divisions of the infected cells, and there are usually multiple infections, that the large warty outgrowths take place.

When *Taphrina aurea* penetrates the poplar leaf, the mycelium remains confined between the cuticle and epidermis, only sending short branches down between the cells of the latter. These are stimulated locally (Plate XII, fig. 30) and become two or three times the normal length, often dividing when they reach full size. There is little disturbance of the rest of the tissues.

Gall reaction to systemic infection.

At the extreme other pole from these cases of strictly localised parasites stand the diseases of the virus group. In many of these the morbid principle appears to be distributed throughout the whole plant. Few produce tissue reactions of the type discussed in this paper, but some do, *e.g.* the curly top of beet and Fiji disease of sugar-cane. In the woody disease of the passion fruit (*Passiflora edulis*), material and sections of which have been kindly provided by Dr R. J. Noble, who has studied it in Australia and proved it to be juice infectious (25), one of the main symptoms is a thickening of the pericarp, which may become

double the normal breadth and show irregular protrusions. The pericarp is abnormally hard and woody, due to extensive thickening and lignification of the cells. In the normal fruit (Plate XII, fig. 31) there is a single epidermal layer, then a hypoderm of three layers of thin-walled cells, below which is a band of lignified sclerotic cells also about three layers deep, followed by the ordinary thin-walled parenchyma of the main mass of the fruit wall. In the woody fruit (Plate XII, fig. 32) the sclerotic band becomes broader and more irregular, while the cells of the underlying parenchyma are also transformed in great part into lignified stone cells. At a later stage (Plate XII, fig. 33) cork forms below the sclerotic band, the outer layers of which (together with the epidermis) may become exfoliated. The phellogen develops in elongated cells, which divide as in the wound cork formation in leaf spots described above, but the cells on the side furthest from the surface become transformed into a sclerotic phelloderm, which shades off into the sclerotic tissues below. The local hypertrophies are due to this sclerotic phelloderm. In the well-known stony condition, termed lithiasis, in pears a similar sclerotic phelloderm is often formed, and several other cases of the kind will be readily recalled (one in the papaw is very familiar to residents in the tropics), but, so far as I know, this is the first that has been ascertained to be the result of a disease of the virus group.

That fungi and insects or mites can produce new structures in plants is undoubted, if by "new" we mean such as are not normally found in the life of the plant. The erineum hairs resulting from mite infestation, or the rosette cells of the epidermis around a *Synchytrium endobioticum* infection, are not like anything found elsewhere in the plant, but have a quite distinctive form. It is noticeable, however, that most of these cases occur in cells which, through the action of the stimulus, have become to a greater extent isolated from the neighbouring tissues than usual. Complicated correlation factors are probably involved, in which the stimulus of the foreign organism is, perhaps, very indirectly concerned. In the great majority of cases there is nothing new in the tissues of reaction, but merely a disorganisation or an intensification or inhibition of the normal processes of tissue formation and differentiation. The insect or fungus can do no more than call out powers which the cells and tissues already possess, powers which plants can ordinarily make use of in meeting such incidents of their life as may necessitate the healing of an injury, or the making provision for coping with unsatisfactory or unduly enhanced supplies of nutriment or light, or other

environmental vicissitudes. Not only the meristems have the ability to respond by the development of new tissues or by modifications in the products of their activity: other living tissues can play a part not less important. It may be that there are some tissues that react more readily than others: the cambium certainly does so, and perhaps the pericycle: but the examples cited above are sufficient to show that these differences must not be exaggerated. As Küster says: "In all living plant cells, even in the cells of the higher plants, there slumbers the potentiality for the development of all histological characters that appertain to the particular species." Many unnecessary complications of interpretation and perhaps some false analogies, in respect of the processes of morbid anatomy, can be traced to an insufficient recognition of this truth.

Besides the acknowledgments already made in the text to those who have been kind enough to communicate specimens and microscopic preparations, thanks are due to Mr L. A. Boodle, of the Royal Botanic Gardens, Kew, for much helpful criticism and advice.

Summary.

The anatomical modifications in the tissues of plants caused by the action of gall-inducing fungi and insects are illustrated by a number of examples, and compared with those produced by various processes of regeneration and wound healing or by factors that lead to anatomical changes without the intervention of wounds, such as nutrition, humidity, and the like. It is concluded that all living cells have the potentiality to react to various stimuli by hypertrophy, hyperplasy, or the development of meristematic tissues, and that the pre-existing meristems are not, of necessity, primarily implicated in gall formation.

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EXPLANATION OF PLATES VIII—XII

PLATE VIII.

Fig. 1. Medullary cavity in sunflower stem inoculated with crown gall by a needle wound in the young flower head, showing vascular ring, cortex, and epidermis formed around the wound. At *x* glandular hairs; at *t* an incipient tumour. (After E. F. Smith.)

Fig. 2. Detail of wall of cavity in Fig. 1, showing cortical tissue, epidermis and hairs. (After E. F. Smith.)

Fig. 3. Callus and wound wood formation in tea canker.

Fig. 4. Commencement of wound cork formation in apple twig inoculated with *Nectria galligena* 11 days previously. The cells outside the brown zone in the cortex and below the pericyclic fibres are elongating and dividing.

Fig. 5. Later stage of wound cork formation in apple twig inoculated with *Nectria galligena* 37 days previously. The wound is completely occluded.

Fig. 6. Citrus scab on rough lemon (*C. limonia*). Early stage showing elongation and division of mesophyll cells.

Fig. 7. Later stage of same, showing cork barrier completely cutting off the necrosed tissue. The original walls of the mesophyll cells are thickened and intercellular spaces almost absent. (Figs. 6 and 7 from sections by H. S. Cunningham.)

PLATE IX.

Fig. 8. Woody cylinder in rooted petiole of *Torenia asiatica* leaf bearing a shoot.

Fig. 9. Earlier stage of formation of same, showing renewed cambial activity in the leaf trace and new cambium forming in the ground-tissue cells on each side, curving round to unite at the top where two cells have only just begun division. The new cambium has formed rows of tracheids inside and phloem elements outside. (Figs. 8 and 9 after Winkler.)

Fig. 10. Petiole of *Chrysanthemum frutescens* about 11 weeks after an inoculation lower down in the stem with crown gall, showing leaf trace transformed into a closed woody ring, in the centre of which is a group of tumour cells in continuity with a strand of similar cells in the protoxylem region down to the point of inoculation.

Fig. 11. Early stage of similar leaf trace, showing new cambium forming on the right-hand side in the ground-tissue cells.

Fig. 12. Later stage from same petiole as Fig. 11, but lower down, showing new woody ring almost complete. (Figs. 10 to 12 after E. F. Smith.)

Fig. 13. Secondary crown-gall formation in sweet-pea stem inoculated lower down about 7 weeks previously, showing closed woody nodule in the cortex.

Fig. 14. Two subepidermal intercellular spaces infected with *Bact. tumefaciens*, showing hyperplasy of surrounding cells to form a more or less concentric sheath. (Figs. 13 and 14 after Riker.)

PLATE X.

Fig. 15. Tangential section of tobacco stem crown gall, showing hyperplasy of cells of cortex to form tumour tissues. At *Cr.* crushed cells; *E.* epidermis.

Fig. 16. Tangential section of same tumour as last deeper in, showing tumour tissue in wood and commencement of extension vertically. *Tr.* tracheids. (Figs. 15 and 16 after E. F. Smith.)

Fig. 17. Early stage of crown gall of black raspberry (*Rubus occidentalis*), showing hypertrophy and hyperplasy of cell layers between cork and pericyclic fibres. Vascular region unaltered. On the left the cortex has begun to split above the phellogen. Note absence of tumour tissue nests.

Fig. 18. Later stage of same, with all the tissues down to the cambium involved. Outer part of xylem broadened below tumour by increase in the medullary rays, and in this part there are no large vessels. Note the bending up of the pericyclic fibres on the right, from a proliferation of the phloem tissues.

Fig. 19. Longitudinal section of swollen cacao shoot from the witches' broom caused by *Marasmius perniciosus*. The primary cortex of the normal part (on the right) has been bent up and crushed by a pericyclic proliferation in the thickened part. Hyphae were found in the pericyclic region of the thickened part near its point of origin. Further to the left the phloem is also much enlarged.

Figs. 20 to 23. Progressive early stages of gall formation on *Acacia decurrens* by the rust *Uromycladium notabile*. In Figs. 21 and 22 the thin-walled phloem hypertrophy is visible, in Fig. 23 the wavy dark line about the centre of the gall represents the position of the cambium, but there is little difference in the cells on each side of this.

PLATE XI.

Fig. 24. Woolly aphis gall on apple twig, showing parenchymatous xylem becoming normal again at the periphery. Tissues outside the cambium not affected. (From a section cut by S. P. Wiltshire.)

Fig. 25. Tea canker, showing wound wood simulating an annual ring. Note its looser structure and scarcity of fibres, except at the periphery where it becomes more normal.

Fig. 26. Longitudinal section of *Veronica chamaedrys* affected by *Sorosphaera veronicae*, showing gall formation in the procambial region and extending out into a leaf.

Fig. 27. Detail of same in the stem, enlarged to show tumour tissue restricted to the procambial region and not extending into the cortex or pith. (Figs. 26 and 27 from sections communicated by E. J. Schwartz.)

Fig. 28. Section of dandelion leaf showing the large bundle on right infected with *Protomyces pachydermus* and that on left free. The fungus is confined to the phloem. (From section cut by W. R. I. Cook.)

PLATE XII.

Fig. 29. Tangential section of dandelion leaf infected with *Synchytrium taraxaci*, showing two large host cells, each surrounded by a capsule of small rectangular cells without intercellular spaces. In the angle of the vascular strand on the left is a surface view of an earlier stage of capsule formation around a host cell below the level of the section. At this depth most of the capsule comes from the mesophyll tissue. (From a section cut by W. R. I. Cook.)

Fig. 30. *Taphrina aurea* on poplar leaf. Early stage, showing elongation of a group of epidermal cells, one of which has divided.

Fig. 31. Normal fruit wall of passion fruit.

Fig. 32. Fruit wall of passion fruit affected by "woodiness," showing the increase of the normal layers of sclerotic cells and the transformation of the parenchyma below into stone cells.

Fig. 33. Later stage of "woodiness," showing the development of phellogen cutting off a sclerotic phelloderm below. (Figs. 31 to 33 from sections communicated by R. J. Noble.)

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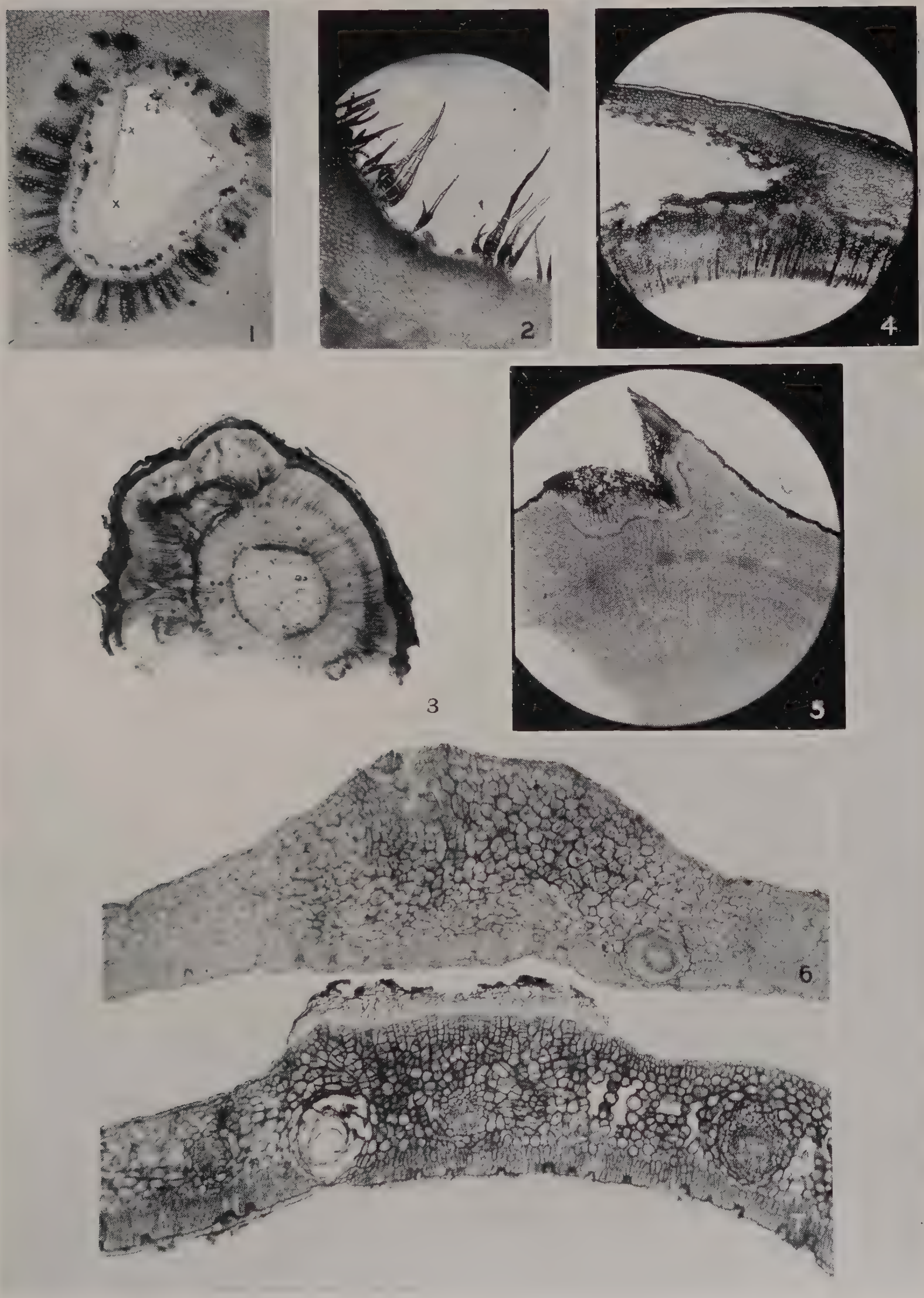
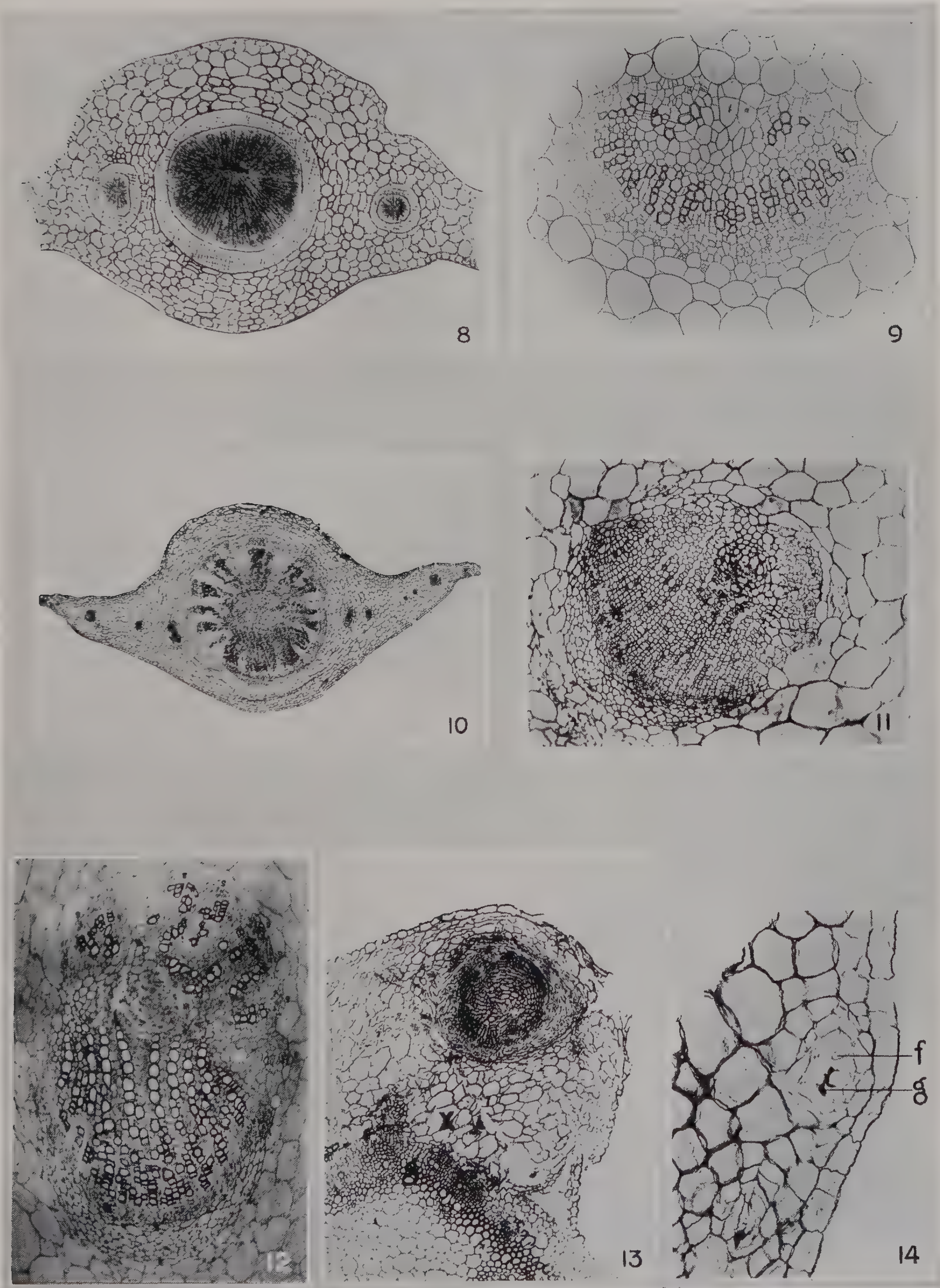


BUTLER.—SOME ASPECTS OF THE MORBID ANATOMY OF PLANTS (pp. 175–212).



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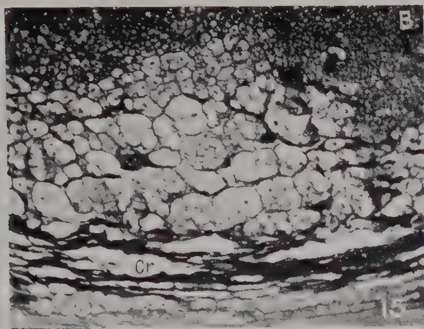

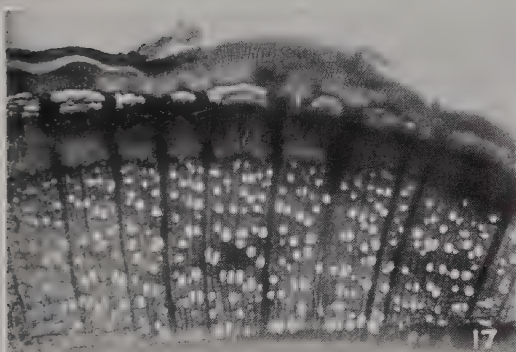

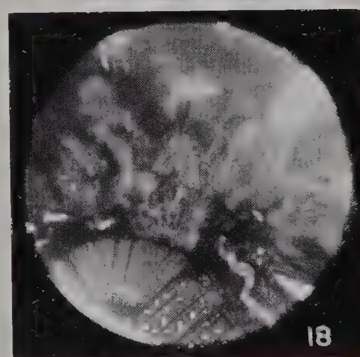

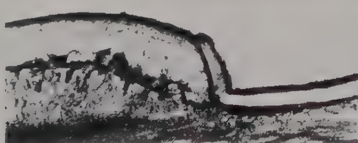
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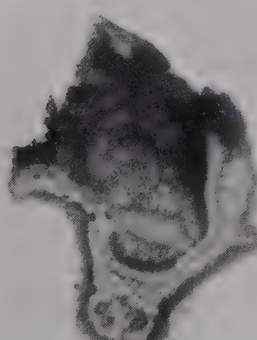
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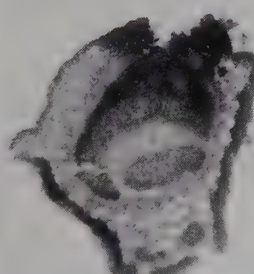
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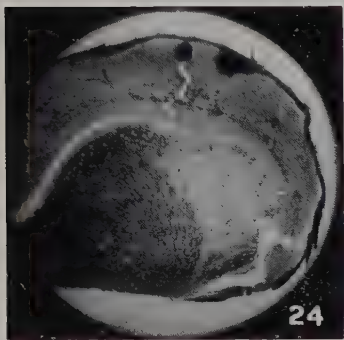

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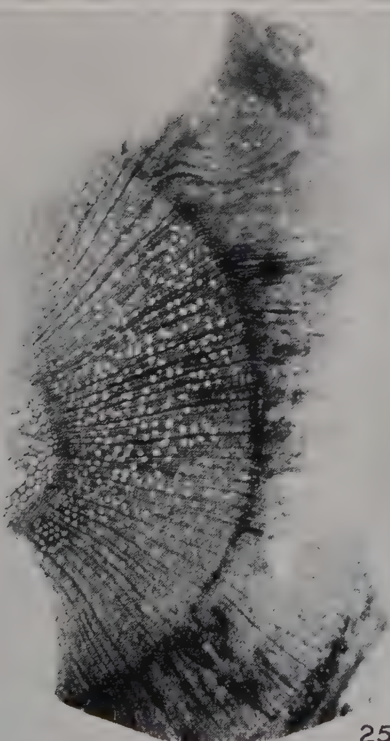

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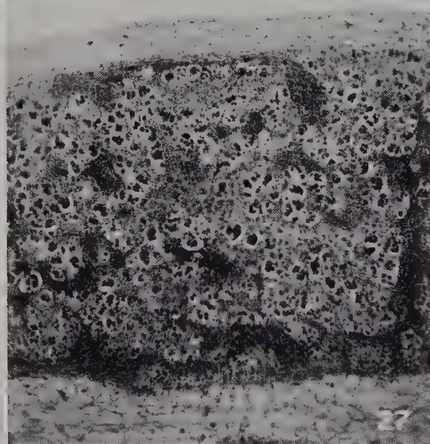

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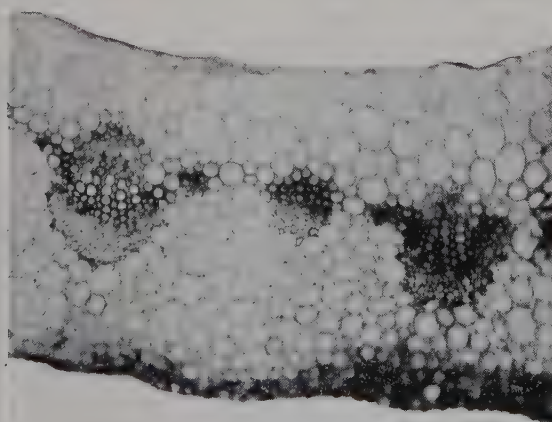

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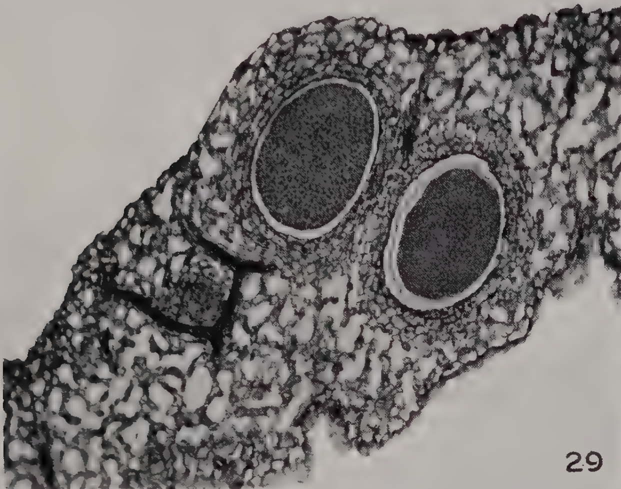
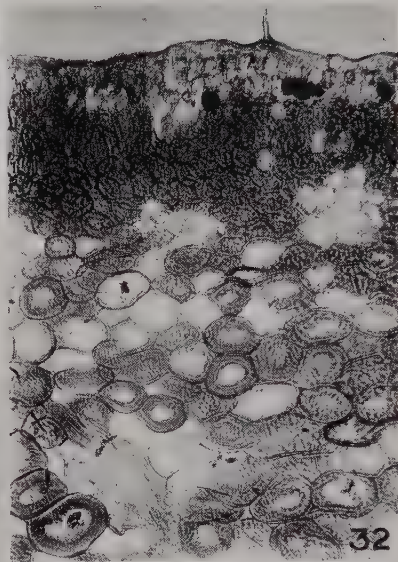
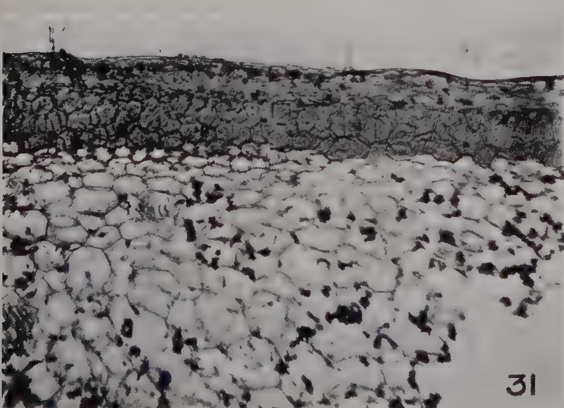
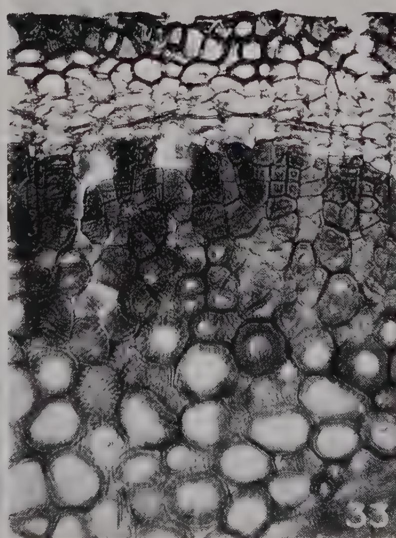
BUTLER.—Some Aspects of the Morbid Anatomy of Plants (pp. 175–212).









BUTLER.—SOME ASPECTS OF THE MORBID ANATOMY OF PLANTS (pp. 175–212).

INTRACELLULAR INCLUSIONS IN MOSAIC OF *SOLANUM NODIFLORUM*

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(With Plates XIII–XVI and 1 Text-figure.)

In the course of a study of the aucuba or yellow mosaic of tomato, inoculation of the virus was made into an extended series of different host plants, and in the examination of these hosts search was made for the presence or absence of abnormal intracellular inclusions, and in particular for bodies of the amoeboid type called by Miss Goldstein X-bodies. The latter were readily found in every plant in which obvious symptoms developed, but were not found in hosts which did not take the disease. In the different hosts they presented minor differences of size, shape or apparent structure, but in all were sufficiently alike as to leave no doubt that they were in every case structures of the same nature. In one host, however, viz. *Solanum nodiflorum*, they were exceptionally conspicuous in the cells of the leaf-hairs, and as in this plant the X-bodies ordinarily occur unaccompanied by those other abnormal inclusions (striate material, crystal plates or packets, and the like) which, in many hosts, *e.g.* tobacco, crowd the cells and complicate the picture, it seemed to offer an unusually favourable opportunity for their more detailed examination. In the following pages are recorded some of the results of this investigation.

The virus of aucuba mosaic of tomato is closely akin to the classical virus of tobacco mosaic, from which it differs only in the greater brilliance of the symptoms it commonly produces (Henderson Smith (8)). In *Solanum nodiflorum* it causes a typical mosaic disease with the characteristic irregular mottling of the leaves (Plate XIII, fig. 1), which is readily transmitted by juice inoculation and in young actively growing plants regularly develops after a short incubation period of 5–12 days.

In this plant the hairs of the leaf are two- to four-celled structures, with rather rigid walls studded with numerous minute papillae (Plate XIII, fig. 2). They stand out stiffly from the epidermis, and along the margins of the leaf project in such a fashion and such numbers that

each individual hair can readily be examined under high powers of the microscope when mounted simply in water or other suitable medium. The cells of the normal hair show nothing but the nucleus, the peripheral cytoplasm and a varying number of strands of streaming protoplasm, with occasionally a few crystals and perhaps a little granular or amorphous matter of uncertain nature in the vacuole, which occupies the bulk of the cell. There are no chloroplasts. In the hairs of an infected leaf the cells contain in addition a large abnormal inclusion, the X-body (Plate XIV), which is never found in normal hairs, and has not been seen in the cells of any plant suffering from any pathological condition other than virus. There is often present also a large spike, which usually lies in the long axis of the cell, as if it were suspended in it, and appears to be crystalline. It can sometimes be seen to be made up of a bunch of hair-like crystals, especially distinguishable at the ends and is sometimes thicker in the middle, producing a slightly tapering appearance. There may be more than one such spike, and these may either be quite separate from one another or may approximate at one end so as to produce a radiating appearance (Plate XVI, fig. 1). They have no connection with the nucleus, and, although sometimes apparently related to the X-body, often are quite separate from it. Their appearance and position are often such as to suggest that they are crystalline formations either of or in cytoplasmic trabeculae.

Typically the X-bodies are roughly spherical (Plates XIV, XV), and usually there is a tendency to a rounding of the contours, whatever the shape. Often, however, they are quite irregular in outline and when in contact with the cell wall or septum are frequently flattened on that side. The smaller bodies, while tending also to rounding, are more usually irregular than the larger, and may appear, especially when lying along the cell wall, as elongated lumpy masses or as thin and rather flat. When very small, it is difficult to feel certain that they are really small sizes of the larger bodies. The size varies considerably. The large spherical bodies may reach $30\,\mu$ in diameter; the small may go down to $5\,\mu$ or less in their larger diameter; in five adjacent epidermal cells they measured 12·4, 12·2, 10·3, 8·5 and $9{\cdot}3\,\mu$ respectively. Even the larger bodies are partially translucent, and are tinted brown or pale yellow; the small bodies may show no colour, perhaps because there is not enough depth of substance to show it. In structure they are coarsely granular. In many cases, indeed, they look as if they were aggregations of smaller particles rather than truly homogeneous, and at the margins of the larger bodies, especially in fixed material, there may sometimes

be seen small projecting particles which confirm this impression (see Plate XIV, in the two terminal cells). But the appearance in this respect is not constant. Some bodies look much more homogeneous than others, *i.e.* are very finely granular. This is particularly well seen in the flat epidermal cells of the leaf, examined in the living state. There one may see in one cell a coarsely granular body, in which vacuolation can be made out, if at all, only with difficulty, and in an adjoining cell, whose margins are contiguous with the first, the body may be very finely granular and homogeneous, with conspicuous vacuoles (Plate XV). In the hair-cells, in the fresh preparation, vacuoles are not conspicuous in the coarsely granular bodies but careful focussing usually shows that they are present in its substance: and in fixed preparations, especially when stained with methylene blue, the vacuolation is usually quite evident. There may be only one or two vacuoles, but the number varies, as many as nine have been counted in one body (Plate XVI, fig. 3). In the coarsely granular type there is usually no sign of a bounding wall or membrane. Sometimes, however, such a membrane or skin does seem to cover part of the surface, and in the more homogeneous forms the suggestion of a skin over the whole or the greater part of the surface may be very strong. No membrane has been seen in *S. nodiflorum* so definite as that figured by Goldstein in tobacco ((1), Fig. 3, p. 563).

In most cases the body is found in close contact with the nucleus, sometimes alongside it, often more or less enveloping it, never incorporated with it. In living cells the body has on more than one occasion been seen to impinge on the nucleus, producing in it a temporary indentation; and both nucleus and body can frequently be seen to move within the cell and quite independently, sometimes drawing apart, sometimes approaching one another, the movements of both being similar and without any suggestion of being autonomous. In fixed preparations the relative positions depend on what happened to be the situation at the time of fixation, and in many instances the two are widely separate. There is, however, as will be explained later, a tendency for the body to be formed, and to remain, near the nucleus, and close juxtaposition is the rule. Within the cell the body may occupy any position, but is usually towards one or other end, most frequently the proximal end. It is not unusual to find in adjacent cells of the same hair two bodies lying one on each side of the same septum. Actual continuity of the bodies has not been demonstrable in such cases. Similar appearances in other plants have been taken as evidence that X-bodies are capable of passing through cell-walls (Likhité (6)), and it may be that this is possible;

but other interpretations may obviously be given and no instance of such passage has been observed in the living tissues. All the cells of a hair may contain one or more bodies, or only one or two of the cells. In the latter case, it is most usual to find them present in the cells nearest the base and absent in the more distal cells, but rare instances have been seen in which the basal cell had none and the more distal cells contained them. The largest bodies are found in the basal cell and the cell next to it, those in the more distal cells being smaller. Since the morbid agent must pass up the hair from the base towards the tip, this might be interpreted as due to the longer duration of infection in the basal cells, but it seems to be generally true that the larger the cell, the larger the body it may contain, and this is in agreement with the experience of others (*e.g.* Kunkel in mosaic of maize (5), Goldstein in mosaic of tobacco (1)).

In *S. nodiflorum* X-bodies show a pronounced tendency, not recorded for the similar bodies described in other plants, viz. a tendency to crystallisation. In large bodies this can often be clearly seen at the surfaces, where definite crystals project from the mass and are very visible at the margins (Plate XIII, fig. 3). But they are usually best seen in the elongated forms lying along the wall of the cell. There the mass can often be seen to be quite certainly partially crystallised, sometimes as fully formed crystals, sometimes as semi-crystalline forms with faces and angles on part only of their surface, such as are frequently seen in crystallising protein. The tendency is accentuated in certain fixing solutions, *e.g.* crystallisation is much more pronounced in material fixed in Bouin than in Carnoy; but it occurs in unfixed material and can be seen in cells where the continuance of protoplasmic streaming shows that the cell is still living. It is most evident in old leaves and in leaves in which infection has been of long duration.

The X-bodies may occur in any part of the leaf, *e.g.* the palisade or the cubical parenchyma cell: they are sometimes beautifully seen in the epidermal cells lying along the veins on the under-surface (Text-fig. 1). The most satisfactory method of demonstrating them in fixed material is first to stain the nuclei red by Feulgen's method, and then counterstain with suitable dyes, such as methylene blue or aniline blue. The nucleus is thereby clearly differentiated from the body, even when partially embedded in it, and the vacuolation of the body is clearly brought out. As a rule they are present in largest numbers in tissues which are macroscopically chlorotic, but they are often to be seen in regions or even in leaves where there is no obvious chlorosis: in the

fern-leaf type of leaf in tomato, for example, they are conspicuous in the hairs (Plate XVI, fig. 4). In *S. nodiflorum*, in a leaf detached from the plant when it is beginning to show local signs of the disease, there may at first be no bodies present in the hairs of the yellow or any other part of the leaf, but later examinations of the same leaf (kept meantime with its petiole in water or nutrient solution) show the bodies appearing progressively in the hairs all round the leaf, not only in the yellow regions but in areas where there is no macroscopic evidence of disease. The relationship between symptoms and the development of X-bodies, however, requires, and will receive, further investigation.

A number of chemical and other tests have been made on these X-bodies in the attempt to get a clearer idea of their nature. These

Text-fig. 1. X-bodies in the epidermis above a vein; stained with Feulgen and methylene blue. The round or elongated unshaded bodies are the nuclei; the shaded vacuolate bodies are the X-bodies.

have been carried out mostly on the hairs *in situ* on a portion of leaf, because of the ease with which the processes can be watched under the microscope. Unfortunately the cuticle of the hairs is highly impervious. Reagents of most kinds enter the cells only slowly, and one usually finds that penetration takes place very unequally in different hairs, and even in different cells of the same hair, and may take place more rapidly through the base of the hair than directly through its walls. In most cases the results have been confirmed by treatment of sectioned material, both of the hairs and other tissues.

The X-bodies withstand boiling in distilled water for 20 minutes, and are not dissolved in alcohol of any strength, in acetone or in chloroform (though in the last they sometimes appear to lose compactness). Heated in a platinum crucible till charred black, the hairs retain their outline perfectly, but the bodies disappear. In $2{\cdot}5\,n$ KOH or NaOH

solution, the bodies and the crystal spike dissolve rapidly, usually leaving no residue but occasionally a granular heap remains in the situation of the body. In stronger alkali, *e.g.* $5\,n$, disappearance may be extremely rapid, both in fresh and fixed (Carnoy, Bouin) material. In strong sulphuric acid and concentrated hydrochloric acid, solution is also rapid; in 75 per cent. HCl, solution may take 15 to 20 minutes but is complete; in 50 per cent. HCl the bodies, even when partially crystallised, do not dissolve in 19 hours at room temperature, nor do they dissolve in strong acetic acid. In sodium hypochlorite they dissolve rapidly. No digestion was obtained with taka-diastase, but probably penetration did not occur.

Millon's reaction. The bodies in fresh preparations turn brown to red-brown; after Carnoy fixation they were definitely brick-red, the colour being deepest where the body is thickest; and in cells where the body had developed definite crystals these also turn red; after Bouin fixation, bodies and crystal forms are red. The colour is intensified and its development accelerated by warming. In no case did the bodies dissolve. In the similar bodies of *S. nigrum* Millon gives a very pronounced red-brown colour.

Raspail's reaction. The leaf portions were left in a concentrated solution of saccharose for 3 hours or more, the solution then drained off and the preparation mounted in strong sulphuric acid. In fresh preparations, the bodies dissolve quickly, turning bright red as they do so, and the red colour diffuses through the cell from the body. After Carnoy fixation, the body may remain undissolved for a quarter of an hour or more, turning bright red; on solution of the body the colour remains localised. After Bouin fixation, no red colour was obtained, although solution was sometimes very slow. In sections of hairs, solution was almost immediate and no red colour was observed; in sections of palisade tissue, a brown-red colour was got before solution.

Biuret reaction. Fresh portions of leaf were placed for 3–4 hours in saturated solution of copper sulphate, washed well in distilled water, and mounted in $2{\cdot}5\,n$ KOH. The bodies turned rose-pink in a few minutes and then yellow. After about 15 minutes dancing particles appear in the cell, the long crystal quickly disappears. Colourless, spherical, rather large droplets appear in the cell, which later become granular and are merged in the protoplasm which contracts from the walls. Then the body progressively breaks down into a mass of granules, which persist for some hours. After Carnoy fixation the whole body turns pink at once, and rapidly disappears. After Bouin fixation, it turns pink, and then yellow.

Xanthoproteic reaction. Tested on sections of palisade tissue, the body turns brown on addition of ammonia (not yellow).

Prussian-blue reaction. The leaf portion was kept in the ferrocyanide solution overnight, washed well in 60 per cent. alcohol, and then the ferric chloride added. Even after 2–3 hours, results are very irregular. In many hairs there is no staining at all, but in some the bodies are deep blue. It is the same after Carnoy fixation; after Bouin fixation, most bodies are a strong blue colour.

Cinnamic aldehyde. The preparations were left 48 hours in 1 or 5 per cent. solution in 60 per cent. alcohol, before the sulphuric acid was added. Bodies turn strong yellow with a tinge of red in both Carnoy and Bouin preparations; pale yellow in unfixed material. Anisaldehyde produced immediate reddening of the bodies. Salicylic aldehyde and vanillin gave no definite results, only a transitory colour developing. With these substances it is difficult to be certain that penetration occurs in the hairs. In sections of hairs, with strong sulphuric acid, vanillin produced a definite mauve-pink in the bodies; salicylic aldehyde gave no colour.

In view of the fact that potash starvation produces in the leaves of some plants a mottling not wholly unlike the mottle of mosaic, it was of interest to ascertain whether there was any indication of concentration of potassium in the X-bodies; and tests were made by the Molisch-Maccallum method (sodium cobalti-nitrite, followed by sulphide) on hairs that had been fixed in Carnoy. The cytoplasm throughout showed many very small black granules, and these were also present in the X-bodies in approximately the same proportion: the bodies did not as a whole turn black. It would seem, therefore, that potassium occurs equally in the cytoplasm and the bodies.

Iodine stains the bodies brown or yellow, not blue nor black. Sharlach R and Sudan III produced no red colour in them, even when penetration had certainly occurred. With osmic acid, the bodies turned brown but not black even after 24 hours in 3 per cent. solution. With material fixed in potassium bichromate, chromic acid and 2 per cent. osmic acid for 48 hours, then thoroughly washed in running water for 12 hours and, after repeated washings in distilled water, kept in 2 per cent. osmic acid for a week at 30–35° C., the bodies were a dark brown but did not have the characteristic black colour of fatty material.

When the living hair, mounted in water, is examined in polarised light, no pleochroism is observed in the cell walls, the crystal spike or the X-bodies. With crossed Nicols the cell walls and septa extinguish

on rotation, but neither the spike nor the bodies nor the crystals into which the body may have resolved appear distinctly at all. Sometimes a doubly refracting crystal is to be seen lying on the surface of the body, and sometimes one or two small crystals appear, embedded in the surface of the body. By ordinary light, the bodies, mounted unstained in Canada balsam or in xylol, are almost invisible; *i.e.* they have a refractive index of about 1·52.

From these various reactions it is evident that the X-bodies, like the striate material and crystal packets investigated by Klebahn (4) in other hosts, are proteid in nature. The fact, established by Holmes (3) in *Hippeastrum* mosaic, that they contain mitochondria suggests that they are protoplasmic in nature; but no nuclear material has been demonstrated in them in *S. nodiflorum* or in any other host. It is unnecessary here to recapitulate in detail the appearances which have led some observers to believe that they are independent living organisms. They are described in the papers by Kunkel (5), Goldstein (1, 2), Likhité (6) and others; and are discussed by the present writer in a paper shortly to be published in *Biological Reviews*. In the hairs of *S. nodiflorum* it has been found possible, by following up an observation made by Miss Sheffield in this laboratory, to watch in individual living cells the development of the X-body from its early beginnings to complete formation[1]. It appears that soon after the virus enters the cell tiny plastic particles appear in the circulating cytoplasm and are carried along in its stream. These particles gradually increase in size, and tend to pause in their course at the junctions or anastomoses of the cytoplasmic strands, before adaptation of the shape of the particles and adjustment of the strands allow them to proceed. During such a pause one particle may be joined by another, and when movement is resumed, the two may either separate or may go on as one united mass. In this way larger and larger masses are built up, until they can be recognised as undeniable X-bodies. There may be several such bodies in the one cell, arising independently and remaining distinct, or they may unite to form one single X-body. The composite bodies may again break apart into two or more smaller bodies, and in this way appearances are presented which might suggest fission; and not infrequently, when a smaller mass joins, or breaks away from, a larger one, appearances occur which simulate pseudopodia and have been so interpreted. There is, however, no division in the sense of multiplication, and the separated masses may

[1] A preliminary account of this work has already been published (Sheffield and Henderson Smith (7)), and a more complete account will be given later.

again unite. The movement is always passive: there is no suggestion of autonomous movement. Both the nucleus and the body may continue to move similarly and independently of one another, even in cells which contain only one large X-body where the process of formation is apparently complete.

This mode of formation accounts for the coarse granularity already described as frequently observed in the bodies of the hairs or epidermis. They are actually aggregates[1] in their earlier stages, but when the aggregated particles have been in contact for a time, they seem to fuse together into a more homogeneous mass, in which vacuolation is distinct. It explains also the tendency of the bodies to be found in close association with the nucleus, because it is there that the cytoplasmic strands meet, and it is in such situations that the bodies tend to form. The nature of the small particles is still undetermined. They give the impression of being foci where the cytoplasm has condensed or consolidated; and, if so, their protein nature and mitochondrial content are intelligible. The body itself should be regarded as a product of the reaction produced in the cell cytoplasm, and it may be that each of the tiny particles is evidence of a local reaction to an ultra-microscopic particulate virus embedded in its substance.

Grateful acknowledgment is made to Dr Margaret Madge for repeated assistance, especially in the cutting of many sections, and the carrying-out on them of various chemical reactions; also to Miss M. M. Browne for the skill and care given to the many plants used in this investigation.

SUMMARY.

A description is given of the intracellular inclusions found in *S. nodiflorum* after inoculation with the virus of the yellow (aucuba) mosaic of tomato. In this plant these inclusions are unusually conspicuous and can be observed in the living cell with exceptional ease. They are of two main types, crystalline spikes and amoeboid bodies, the latter corresponding to the X-bodies found in other plants. A detailed account is given of their appearance, structure, position in the cells, relation to the nucleus etc. The X-bodies may be either coarsely granular, looking like aggregates of particles, or more homogeneous; and they have a pronounced tendency to crystallise into the forms commonly seen in protein crystals. They are vacuolate, and occur in all the tissues of the leaf.

[1] When a portion of leaf containing well-formed bodies in every hair is mounted in distilled water and evaporation prevented, the bodies may break down again and disappear from the cells within 48 hours.

They give the usual tests for protein (Millon, Biuret, etc.) and are not of fatty nature. Their formation has been followed in individual living cells from the very early stages to their complete development. The mode of formation, viz. by the aggregation of small particles, which are carried in the protoplasmic streaming, is shown to account for the appearances which have led various observers to conclude that they are living organisms or parasites, a conclusion for which no evidence has been found in the present investigation.

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DESCRIPTION OF PLATES XIII—XVI

PLATE XIII.

Fig. 1. Leaf of *S. nodiflorum*, inoculated with aucuba mosaic. Length of leaf: 4 inches.

Fig. 2. Hair of *S. nodiflorum*: normal. Carnoy fixation; unstained; ×310.

Fig. 3. Crystallising X-body in leaf-hair. Bouin fixation; unstained; ×450. Nucleus partly seen at left upper edge of mass of crystals.

PLATE XIV.

Hair showing X-bodies. Carnoy; unstained; ×430. Note the granular appearance; and in the two terminal cells the projecting particles. In the third cell the nucleus is visible at the upper edge of the body.

PLATE XV.

Epidermis. Fixed in Carnoy; stained with Feulgen and methylene blue; ×820. The small dark bodies are the nuclei, the larger bodies the X-bodies. Above the stoma is a cell where nucleus and X-body are not in juxtaposition.

PLATE XVI.

Fig. 1. Hair-cell showing two radiating crystal spikes. Fresh preparation, mounted in water; unstained. Nucleus visible at upper edge of body.

Fig. 2. Section of palisade tissue. Carnoy; Heidenhain and anilin safranin.

Fig. 3. X-body in epidermis of leaf. Carnoy; Feulgen and methylene blue; ×1750. The smaller dark body is the nucleus.

Fig. 4. Glandular hair from fern-leaf in tomato. Fresh preparation; unstained. Note the separation of nucleus and X-body in the cell next the base-cell, and their juxtaposition in the cell above.

(*Received November* 1*st*, 1929.)

Fig. 1.

Fig. 2.

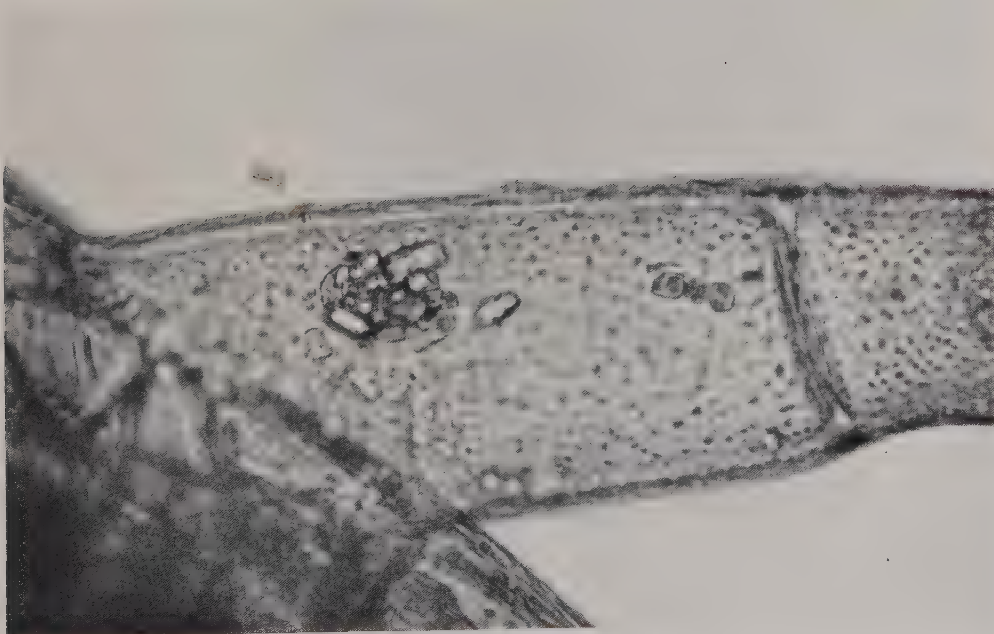
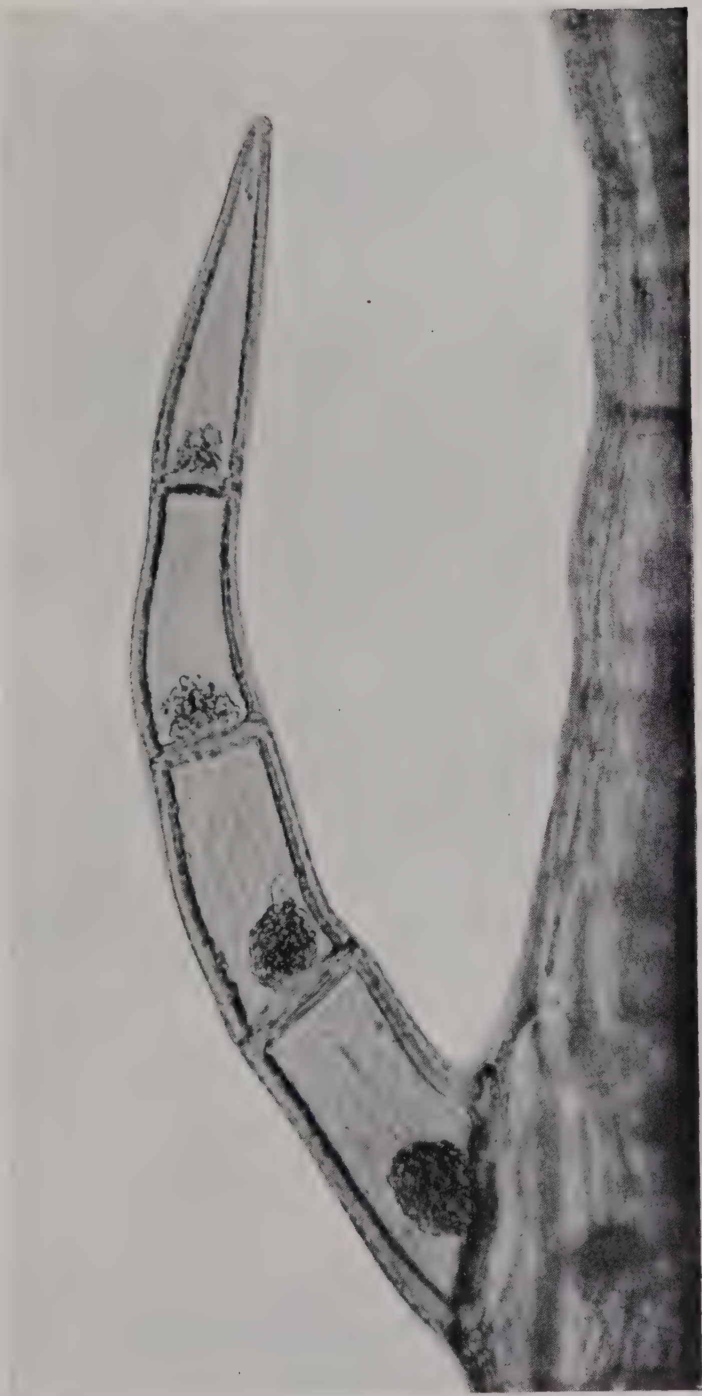


Fig. 3.

HENDERSON SMITH.—INTRACELLULAR INCLUSIONS IN MOSAIC OF *SOLANUM NODIFLORUM* (pp. 213–222).



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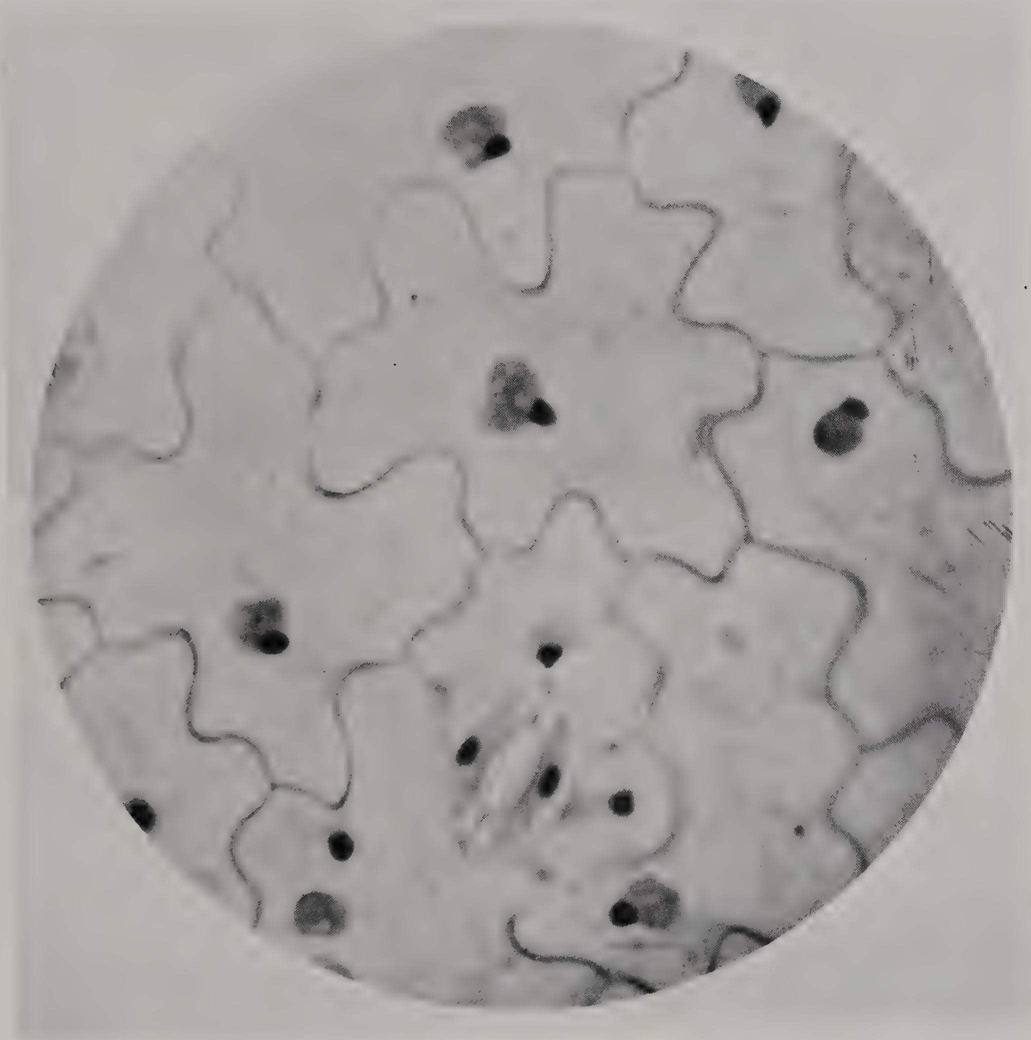

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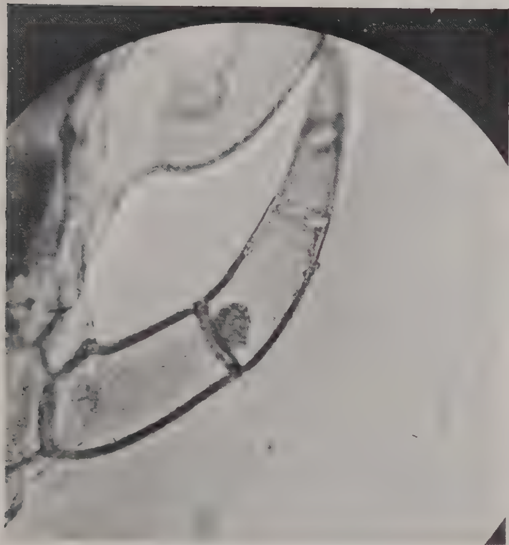

Fig. 1.

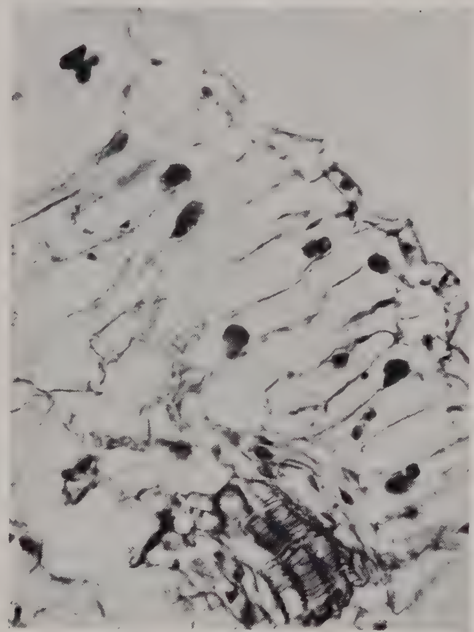

Fig. 2.

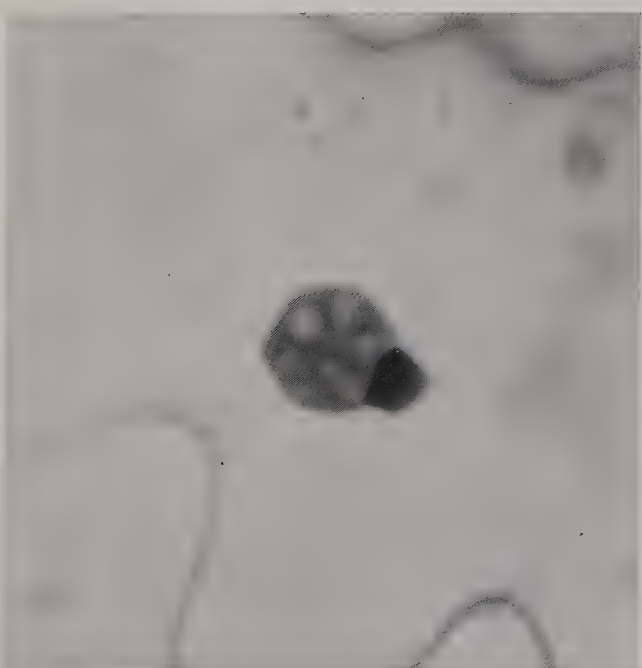

Fig. 3.

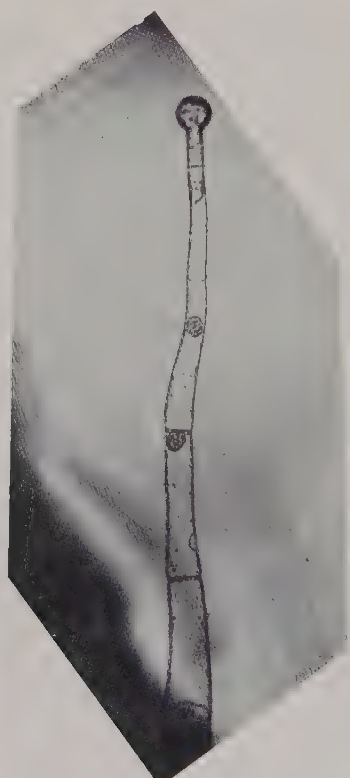

Fig. 4.

HENDERSON SMITH.—Intracellular Inclusions in Mosaic of *Solanum nodiflorum* (pp. 213–222).

STUDIES ON POTATO VIRUS DISEASES

VII. SOME EXPERIMENTS WITH THE VIRUS OF A POTATO CRINKLE WITH NOTES ON INTERVEINAL MOSAIC

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(With Plates XVII–XX.)

CONTENTS.

1. Introduction.

As knowledge of potato virus diseases accumulates, it becomes increasingly evident that there exist in the mosaic and crinkle group, if not many viruses, at least many strains possibly of the same virus of varying degrees of virulence, each of which possesses its own particular type of symptom and behaviour. For example, as the work of Salaman (1) has shown, there is no guarantee that the crinkle disease exhibited by one variety is the same kind of virus as the crinkle exhibited by another unless one plant has been infected from the other. The crinkle virus used in the experiments described in the ensuing paper was obtained from the potato variety Myatt's Ashleaf. These plants had been under observation for two years in the insect-proof glasshouse at Cambridge and exhibited a marked crinkle. The virus of interveinal mosaic was obtained from the variety President kindly given to the writer by Dr R. N. Salaman. This

plant, also, was one of a number of similar plants which had been grown for some seasons under the same insect-proof conditions.

The difficulties in adequately describing a particular crinkle or mosaic disease of the potato are immense, and these difficulties are likely to increase with increasing knowledge so long as the potato virus worker is compelled to describe a specific disease by its symptoms alone. Not only is the symptom expression of the same virus different in different potato varieties, but the symptoms may vary according to the method of transmission. Again, it cannot be said with certainty that the virus itself is not altered, perhaps only temporarily, by varying methods of transmission, or by passage through certain potato varieties.

The experiments described in this communication fall into two sections: the first describes the transmission of the viruses by different methods to several varieties of potatoes, while the second consists of a series of cross inoculations to the tobacco plant and *Datura* sp. and is complementary to the work already carried out by the writer with a potato mosaic (2, 3). The aphis sp. used throughout the experiments was *Myzus persicae* Sulz. which has proved itself the most efficient transmitter of potato viruses. The writer has pleasure in acknowledging the assistance given by Miss M. E. Sewell, during the progress of this work.

2. Transmission of the crinkle virus from Myatt's Ashleaf to healthy potatoes of different varieties by needle.

The symptoms of crinkle as portrayed by Myatt's Ashleaf consisted of a marked mottling of yellow or light green, and darker green together with considerable crinkling and distortion of the leaf margins (Plate XVII, fig. 1). The virus was transmitted by means of needle scratches into the leaves of a number of different potato varieties, the plants being inoculated in batches of three or six.

President. Six experiments were carried out with this variety, involving a total of about 25 plants. Of these, two plants only showed symptoms after a period of 18 days. Instead of crinkle, however, mosaic mottling of a mild type developed. Some further experiments with this "mosaic" are described on p. 237.

Arran Victory. The same experiments were performed with this variety with negative results, except in one case where one plant developed crinkle in 9 days. The symptoms exhibited were of the same general type as were shown by the source of infection.

Big Ben, Up-to-Date. Negative results were obtained with these two varieties.

Great Scot. Four plants out of six of this variety developed crinkle, together with streak lesions 20 days after inoculation. The crinkle symptoms as portrayed by Great Scot differed from those of the source of infection. The leaves were of an unusually deep green, glassy with considerable yellowing of the veins, and some crinkling and distortion. The streak symptoms consisted of large numbers of lesions scattered over the leaves, the leaf-drop type of streak did not develop or only to a very small extent. With continued growth of the plant these lesions tended to disappear.

3. TRANSMISSION OF THE CRINKLE VIRUS FROM MYATT'S ASHLEAF TO HEALTHY POTATOES OF DIFFERENT VARIETIES BY THE APHIS *M. PERSICAE*.

A large series of transmission experiments was carried out with the aphis *M. persicae* from crinkle Myatt's Ashleaf to a number of different potato varieties. The aphides were first colonised on the crinkle Myatt's Ashleaf and then transferred in the usual way to sprouted half tubers planted under glass chimneys. Successful transmission was obtained only to the variety President after 20 days. Four President plants out of eight developed a crinkle, which was milder than that produced by grafting the same virus (see section 4) but more pronounced than that produced by needle inoculation with the same virus. The symptoms consisted of mottling accompanied by slight crinkling of the leaf surface. Negative results were obtained with the other varieties tested, *i.e.* Arran Victory, Great Scot and Kerr's Pink.

4. TRANSMISSION OF THE CRINKLE VIRUS FROM MYATT'S ASHLEAF TO HEALTHY POTATOES OF DIFFERENT VARIETIES BY GRAFTING.

Scions of crinkled Myatt's Ashleaf were grafted on four potato varieties as follows:

President. In 23 days this variety developed a very severe type of crinkle with bold yellow and light green mottling and much distortion of the leaves. This disease was exactly comparable to that shown by the source of infection.

Arran Victory. This variety developed a severe crinkle in periods of from 13 to 23 days, the leaves showed a well-marked mottle of yellow chiefly on the veins and a tendency of the outer edges to draw together underneath, so that an effect of puckering was produced.

Great Scot. In 17 days there developed a fairly severe streak which tended to kill the growing points (Plate XIX, fig. 1). This streak seemed to be more severe than that produced by needle inoculation (see section 2). There was also some mottling but little crinkling.

Kerr's Pink. A fairly strong mottle developed in Kerr's Pink in 19 to 28 days, followed some days later by the appearance of characteristic streak lesions. These remained in the form of large irregular spots and did not develop into the leaf-drop form, nor attack the growing points as was the case with Great Scot.

5. Experimental transmission of the crinkle virus between potato and tobacco and potato and *Datura* sp.

(*a*) *Needle inoculation of tobacco with the crinkle virus from Myatt's Ashleaf.*

The varieties of tobacco used in this work were White Burley and Virginia, and are the same varieties as those employed in the potato mosaic inoculations (2). Young seedlings were inoculated by needle scratch with the crinkle virus; in all about 50 plants of each variety were inoculated, symptoms developing in 5 to 10 days. The disease produced in tobacco by the crinkle virus is symptomatically somewhat similar to that produced by needle inoculation with mild mosaic, but is undoubtedly more severe. The tendency to ring formation in tobacco is also present in this crinkle virus. Occasionally small rings and half rings have developed after inoculation with the crinkle virus, but the more common expression of symptoms is a marked mottle with some necrotic spots and a tendency for the veins to stand out (Plate XVII, fig. 3). There is evidence to show that progressive needle inoculation of the crinkle virus through successive tobacco plants does produce increase in virulence (Plate XVII, fig. 4), though the writer has not yet succeeded in bringing this virulence quite up to the pitch of severity developed with potato mosaic (3), although this is probably only a matter of continuing the progressive inoculation[1]. At the fourth or fifth successive inoculation it is often the case that the veins become yellow and necrotic, while the interveinal tissue appears raised above the level of the veins and rather shiny, giving the leaf a crinkled appearance; in such a plant the mottling is not very evident. Later, a very pronounced mottling together with necrotic lesions may develop, a complex of

[1] Further experiments have shown that a high degree of virulence can be obtained by progressive inoculation through tobacco.

symptoms which closely resembles that produced by a potato mosaic in tobacco after many progressive inoculations.

(b) Return of the crinkle virus from tobacco to healthy potato of different varieties by needle.

Nine varieties of healthy potatoes were inoculated by needle scratch with the virus from tobacco which had itself been infected by needle scratch from crinkled Myatt's Ashleaf. The following potato varieties were used:

(1) President.
(2) Arran Victory.
(3) Arran Chief.
(4) Great Scot.
(5) King Edward.
(6) Big Ben.
(7) Kerr's Pink.
(8) Up-to-Date.
(9) Majestic (grown from true seed).

(1) *President.* Twelve healthy President plants were inoculated in two series of six each. All twelve were infected, symptoms appearing after 14 and 16 days. Mottling in the form of large isolated pale spots appeared on the younger leaves; these rapidly developed into a condition of crinkle exactly similar to the crinkle produced by grafting healthy President with a scion of Myatt's Ashleaf crinkle. At the same time streak lesions appeared on the middle leaves, accompanied by the typical streaks on the veins and stem; this was soon followed by leaf-drop streak, which usually first appeared on the lowest leaves and spread rapidly upwards until only the topmost shoot was left alive, the remainder of the leaves hanging down in the manner shown in Plate XVIII, fig. 3.

It was found that this severe crinkle and leaf-drop disease could be passed on to healthy potatoes by both needle and aphis (*M. persicae*) with the greatest ease, such inoculations by either method being positive in nearly 100 per cent. of the cases.

(2) *Arran Victory.* Twelve plants inoculated, twelve infected, symptoms appearing in 8 to 14 days. The same well-marked crinkle developed as was shown by the Myatt's Ashleaf plant from which the virus was originally taken, together with lesions and some leaf-drop streak, though Arran Victory was definitely more resistant to the leaf-drop element than was President.

(3) *Arran Chief.* Six plants were inoculated and all six became infected, symptoms appearing in 13 days; the same severe crinkle developed followed by the leaf-drop streak.

(4) *Great Scot.* Four plants inoculated, four infected, symptoms developed in 8 days, consisting of a fairly distinct crinkle and leaf-drop streak as in the other varieties.

(5) *King Edward.* Four plants inoculated, four infected, symptoms of leaf-drop streak appearing after 10 days. The usual mottling developed on the younger leaves, and this gradually increased into the crinkle form about 16 days later.

(6) *Big Ben.* Four plants inoculated, four infected, symptoms appeared in 8 days. This was by far the most susceptible variety, and the plant was killed in every case. The development of the disease was remarkably rapid and the symptoms consisted entirely of a virulent type of leaf-drop streak without crinkling or mottling. The symptoms appeared mostly on the lower leaves first, in the form of numerous streak lesions, the leaves then turned yellow, shrivelled and rapidly died, remaining attached to the stem (Plate XVIII, fig. 2). The leaf-drop streak progressed steadily up the plant until finally it reached the topmost shoot which was killed in turn, thus bringing about the death of the plant. This variety showed local symptoms on the inoculated leaves, an unusual occurrence in potato viruses, large numbers of round streak lesions appearing after 8 days, particularly on those leaves which had been inoculated by rubbing (Plate XIX, fig. 2).

(7) *Kerr's Pink.* Eight plants inoculated, eight infected, symptoms developed in 14 days. This variety shows a crinkle which differs somewhat from that portrayed by other varieties of potato. The symptoms take the form of large yellow spots, chiefly at the leaf margins and often on a vein. There is also an indentation at the site of the yellow spot, which causes a distortion of the leaf margin and some slight general crinkling may be present as well. Accompanying the crinkling are numbers of streak lesions on the leaves. The leaf-drop element is less pronounced in this variety and may be absent altogether.

(8) *Up-to-Date.* Four plants inoculated, four infected, acute leaf-drop streak developed in 13 days. The typical streak lesions appeared along the veins of the leaves, followed by severe leaf-drop with total collapse and final death of the plant (Plate XIX, fig. 1). This is interesting in view of the fact that Up-to-Date is well known as a "carrier" of the streak virus. Compare needle inoculations into this variety with the same crinkle virus which had not passed through tobacco (p. 225).

(9) *Majestic.* Six plants of this variety derived from the true seed were inoculated. Crinkle with severe streak lesions which rapidly de-

veloped into the leaf-drop type, appeared in all six plants after 14 to 16 days.

(*c*) *Return of the crinkle virus from tobacco to healthy potatoes by the aphis* M. persicae.

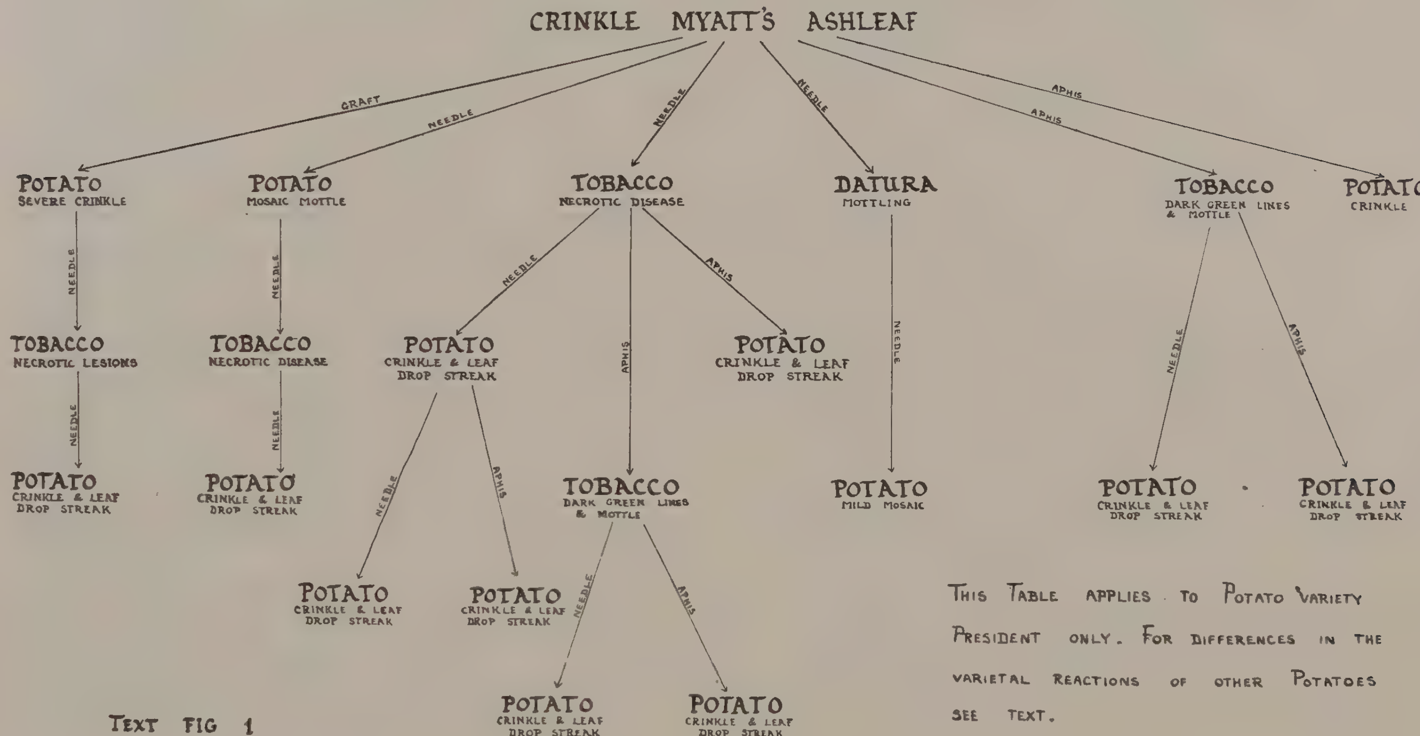
The aphides were colonised in the usual manner upon tobacco plants which had been needle-inoculated with the crinkle virus from Myatt's Ashleaf, and which showed the type of disease illustrated in Plate XVII, fig. 3. They were then transferred to sprouted half tubers or young plants of President and Arran Victory, six of each. All six Presidents and five of the Arran Victory became infected after 18 to 21 days (Plate XIX, fig. 4). The plants exhibited the same symptoms as were produced by needle inoculation from the same tobacco plants. This experiment serves to emphasise the increased infective power of the crinkle virus to the aphis after passage through tobacco. The writer had been unable to infect the variety Arran Victory with the crinkle by means of *M. persicae* before the virus had been passed through the tobacco plant (see section 3), but after this had been effected the aphis transmitted the virus to Arran Victory as well as President.

(*d*) *Aphis inoculation of tobacco:* (1) *from crinkled Myatt's Ashleaf*; (2) *from tobacco plants needle-inoculated with the crinkle virus from Myatt's Ashleaf.*

(1) Eighteen tobacco seedlings, var. White Burley, were inoculated in three experiments of six plants each, with *M. persicae* from crinkle Myatt's Ashleaf. In 10 to 14 days a disease developed symptomatically identical with the disease produced in tobacco by inoculation with *M. persicae* from mosaic Arran Victory. This disease appeared first as a clearing of the veins of the younger leaves, followed by the development of the typical lines and spots of darker green accompanied by a faint mottle (Plate XVII, fig. 2). In no case was it possible to produce with the aphis the brilliant symptoms illustrated in Plate XVII, figs. 3, 4, which followed needle inoculation into tobacco from the same crinkle potato plant.

(2) Twenty-four tobacco seedlings in four experiments of six were inoculated by means of *M. persicae* which had been colonised on a tobacco plant needle-inoculated from crinkle Myatt's Ashleaf (Plate XVII, fig. 1). A disease of mottle and dark green lines developed, apparently identical to that described as a result of aphis transmission to tobacco from crinkle Myatt's Ashleaf in the preceding paragraph.

TRANSMISSION OF A CRINKLE VIRUS BETWEEN PRESIDENT POTATO, TOBACCO & DATURA



THIS TABLE APPLIES TO POTATO VARIETY PRESIDENT ONLY. FOR DIFFERENCES IN THE VARIETAL REACTIONS OF OTHER POTATOES SEE TEXT.

TEXT FIG 1

The percentage of infections in these latter experiments was slightly higher than in those described in the foregoing section. When this disease was returned to healthy President by aphis or needle, crinkle and leaf-drop streak resulted. (See Text-fig. 1.)

(*e*) *Return to healthy potato:* (1) *by aphis*; (2) *by needle of the green lines disease induced in tobacco by aphis transmission from crinkle Myatt's Ashleaf.*

(1) *By aphis.* Four separate experiments of three plants each, involving nine President and three Arran Victory, were carried out. All the nine President plants developed typical crinkle and leaf-drop streak in periods of 17 to 21 days, while two out of the three Arran Victory plants developed a severe crinkle but little streak. This is noteworthy, as the same aphis had failed to carry the crinkle virus direct to Arran Victory from Myatt's Ashleaf, but would transmit it first to tobacco where it produced the faint green lines, pick it up from that plant and then infect Arran Victory and President.

(2) *By needle.* A parallel series of experiments to those described in the preceding paragraph was carried out, but in this case the green-lines disease was returned to healthy potatoes by the needle. Three varieties were inoculated, *i.e.* President, Arran Victory and Majestic (from seed). All three varieties were infected, President and Majestic developed crinkle and leaf-drop streak, the Majestic showing large clear lesions in the leaves as well as the other symptoms. Arran Victory developed crinkle only.

(*f*) *Transmission of the crinkle virus between* Datura stramonium *and healthy potato by needle.*

Twelve plants of *Datura stramonium* were inoculated by needle scratch with the crinkle virus. First symptoms appeared in 10 to 12 days, consisting of a mottling on the younger leaves, which later developed into accumulations of dark green chlorophyll along the veins (Plate XX, fig. 2). The virus was then returned by needle scratch to healthy President plants, out of four plants inoculated three developed symptoms in 14 days. These consisted of a mild mosaic mottling only; with further growth of the plants the symptoms entirely disappeared. In this case the *Datura* seems to have had an attenuating effect upon the virus; whether permanently or not has not been determined.

6. Some notes on interveinal mosaic.

Interveinal mosaic, as exhibited by the variety President from which the virus used in these experiments was taken, consists of a well-marked mottle of very pale green between the veins of the leaf. The colour of the leaves themselves is of a much darker green than that usually found in healthy President potatoes; there is no crinkling or distortion of the leaves.

(*a*) *Transmission of interveinal mosaic to healthy potatoes by means of* M. persicae.

Six half tubers, variety President, and the same number of Arran Victory were infected with the aphis *M. persicae*, which had been fed on a President plant with interveinal mosaic. Symptoms of interveinal mosaic developed in 14 days in three of the President plants but the Arran Victory remained healthy.

(*b*) *Transmission of interveinal mosaic to healthy potatoes by the needle.*

It was found fairly easy to transmit the virus of interveinal mosaic by needle from a President plant affected with the disease to healthy President, other varieties not being used. Symptoms developed in 11 days, appearing first as a close mottling on the younger leaves; the disease as finally portrayed was the same as that exhibited by the source of infection.

(*c*) *Transmission of interveinal mosaic to healthy potatoes by grafting.*

Six plants of healthy President, grafted with scions from plants of the same variety affected with interveinal mosaic, developed the normal disease similar to that shown by the source of infection in periods of 14 to 21 days.

Six plants of Arran Victory, grafted with scions from the same President affected with interveinal mosaic, developed symptoms in 18 days. In this variety, however, there developed a large number of streak lesions on the leaves; later interveinal mottling appeared together with the dark green colour which appears to be characteristic of the disease. The Arran Victory plants finally grew away from the streak lesions, no more of which developed, the interveinal mosaic as finally presented by Arran Victory resembling very closely that portrayed by President.

Three plants of healthy Great Scot, grafted with the same interveinal mosaic, showed symptoms in 17 days. As with Arran Victory, streak spots developed, but in Great Scot these were of a more severe character, taking the form of large clear lesions in the leaves, which later developed into a form of leaf-drop streak.

(d) Transmission of the virus of interveinal mosaic between tobacco and potato by needle.

Six White Burley and six Virginia seedlings were inoculated by needle scratch with the virus of President interveinal mosaic. Symptoms developed in 10 days, and consisted of a spot necrosis with some mottling on White Burley and the formation of small rings upon Virginia. In both varieties the symptoms were very similar to those produced in tobacco by needle inoculation with mild mosaic from Arran Victory (2). After passing the virus on through a few successive tobacco plants, clear double and treble concentric rings appeared on the needle scratches. Needle inoculation was then made from these tobacco plants back into healthy potatoes, vars. President and Arran Victory. In 9 to 11 days all the plants developed a marked mosaic which, however, still retained its interveinal character and abnormally dark foliage. The symptoms were considerably brighter than those exhibited by the virus before its passage through tobacco. Later there developed two additional factors: in both Arran Victory and President large numbers of round streak lesions appeared on the younger leaves (Plate XX, fig. 3), while the leaves of the topmost shoots assumed a decided crinkling and distortion; in other words, President was now affected with interveinal crinkle, together with the concomitant streak lesions. These streak lesions did not appear in President with interveinal mosaic before passage of the virus through tobacco, though they did in Arran Victory, while the crinkling of the leaves appeared also only after passage through tobacco.

The virus of interveinal mosaic, therefore, behaves in a similar manner to that of ordinary mild potato mosaic in its reactions after passage of the tobacco plant. The symptoms become brighter, infective power is increased, and symptoms of streak are developed, together with crinkling and distortion of the leaves. At the same time, in the present case, the symptoms retain their interveinal character and abnormal darkness of foliage.

As the virus of interveinal mosaic is passed on through successive tobacco plants a marked increase in virulence develops. A number of healthy President plants were inoculated with the interveinal virus from

a tobacco plant showing this increased virulence. In 8 to 10 days a virulent streak developed; this appeared first as large numbers of very small lesions which gradually increased in size, coalesced and formed one large lesion, practically destroying the leaf. The interveinal mosaic was entirely subordinated to the streak and in some cases its presence could hardly be detected. It appears that the severity of this streak developing in the potato is directly correlated with the number of progressive inoculations through the tobacco plant.

7. Discussion.

The following points of interest arise from the foregoing study and merit special attention. Firstly, further evidence is offered of alteration in the nature of potato viruses of the mosaic group by passage through plants other than potato; secondly, it has been found that the symptom expression of a particular virus in its plant host may vary according to the method of infection of that plant host; and thirdly, the wide range of difference in the varietal reaction of potatoes to the same virus is emphasised.

As regards the first point, the crinkle virus after passage of tobacco was found to cause a very severe crinkle and leaf-drop streak disease in every potato variety tested, whereas the same virus before passage of tobacco failed to infect some varieties, and caused crinkle either alone or with a milder type of streak in others. Outstanding was the reaction of the varieties President, Big Ben and Up-to-Date to the crinkle virus after passage of tobacco. Big Ben was the most susceptible, and was killed by leaf-drop streak in every case. President was also very susceptible, though less so than Big Ben. The case of Up-to-Date is of interest, as this plant is well known as a carrier of streak, and inoculation of the crinkle virus before passage of tobacco had no effect upon it, but after the virus had been passed through four successive tobacco plants, the Up-to-Dates were killed by a leaf-drop streak comparable to that of Big Ben (Plate XIX, fig. 1).

The aphis transmission of the crinkle virus direct to potato and to potato after being transmitted through tobacco by the same aphis, offers a parallel case to that of the needle. Although the symptoms developing in the aphis-infected tobacco were entirely different from those produced by the needle (Plate XVII, fig. 2), yet the effect of the virus from such tobacco upon healthy potatoes was equally disastrous. In the same way it was found that the aphis was able to infect certain potato varieties with crinkle and leaf-drop streak after the insect had

passed the virus through tobacco, when direct aphis transmission from crinkled potato to the same varieties had repeatedly failed. It has thus been proved that the tobacco plant will act as an almost symptomless carrier of severe crinkle and leaf-drop streak, when *aphis-infected* from a crinkled potato plant, and this fact may explain the rapid degeneration of certain potato varieties in tobacco-growing countries. The practical point arising from these facts seems to be the advisability of growing potato and tobacco crops as far apart as possible.

The phenomenon of increased virulence was particularly well illustrated by the experiments with interveinal mosaic. Passage of this virus by needle, once through tobacco and back to the same variety of potato, produced in that potato a disease which still retained its interveinal character, but was more of the nature of a crinkle than a mosaic and showed numerous streak lesions. The severity of the streak developing in the inoculated potatoes appeared to be directly proportional to the number of progressive inoculations through tobacco. In the last experiments the plants were practically killed by leaf-drop streak, and the interveinal mosaic was entirely suppressed.

This increase in virulence of mosaic viruses appears to be closely connected with the elusive potato virus known as streak. The grafting experiments carried out in these studies with crinkle and interveinal mosaic showed that both viruses contained the streak element which developed only on certain varieties. Alternatively, it may be said that the symptom expression of the particular crinkle virus on certain varieties was in a streak-like form. It may be suggested then that passage of tobacco merely liberates in some way the streak virus which was already present, so that it attacks every potato variety inoculated. If this supposition be correct, then it follows that in the extensive experiments previously carried out by the writer (2) with a mild mosaic of potato upon tobacco, the disease of streak was latent in that virus also, and was liberated by passage of tobacco to attack the potato variety which had previously "carried" it, *i.e.* Arran Victory.

From this frequent association of the streak virus with the mosaic group, it might be suggested that the disease streak does not exist as a separate entity, but is an integral part of every mosaic or crinkle virus and can be brought to the fore under certain conditions. This theory, however, does not explain the increasing virulence of the potato mosaic virus to the tobacco plant itself which is induced by progressive inoculations. An alternative theory is that there are two streak diseases, one, the normal potato streak which may or may not be "carried" by the mosaic

or crinkle potato, and the other, an artificially produced streak developed by some change in the virus consequent upon passage through tobacco, which cannot be "carried" by any potato variety. If this be the case, it is one more illustration of the difficulties of diagnosis by symptoms, as the streak appearing after passage of the crinkle or mosaic virus through tobacco is symptomatically identical with leaf-drop streak as it occurs normally on many varieties of potato.

Another case in point is the effect on the crinkle virus of passage through *Datura* sp. Only one experiment was performed in this case, and for that reason is open to criticism, although the results seemed definite enough. Here, it may be recalled, the virus of crinkle caused a characteristic mottling on *Datura*, and inoculation of healthy potato from such a *Datura* produced only a mild mosaic mottling. Thus the *Datura* appears to have had the reverse effect to that of the tobacco, and to have reduced the virulence of the crinkle virus. It is possible, however, that this apparent reduction is only of a temporary nature. It may be of interest to recall in this connection that *Datura*, inoculated with potato mosaic which had been passed through many tobacco plants, reacted violently to the streak element there and developed large lesions (2). Again the question arises as to the exact cause of the curious ringspot disease which develops in tobacco after needle inoculation with several potato virus diseases. Are these rings due to the virus causing the visible symptoms or to a streak possibly latent within the plant? The writer has now produced in tobacco, three slightly differing types of ringspot by *needle* inoculation with the virus of mild mosaic (Arran Victory), of interveinal mosaic (President) and of crinkle (Myatt's Ashleaf). The whole tendency of these experiments seems to be to emphasise the close affinities existing in the potato viruses, mosaic crinkle and streak. The second point was the production of different symptoms by differing methods of transmission of the same virus. The outstanding examples of this are the comparative transmissions of crinkle and mosaic to tobacco by aphis and needle respectively. These examples, however, are open to the criticisms that the virus may have been changed somewhat by passage through the body of the insect, or that the aphis was separating out a virus complex. The case of comparative transmissions of the crinkle virus to President by needle and grafting, respectively, would avoid this difficulty. Needle inoculation of the crinkle virus gave in one or two cases a mosaic only, while grafting gave a severe crinkle, both in the same variety of potato. That this needle-induced mosaic was one in symptoms only was proved by its

transfer to tobacco, where it gave identical symptoms to those produced by the original crinkle virus, and when returned from the tobacco to President potato again, it gave crinkle and streak. It may well be, therefore, that many potato plants apparently exhibiting a mosaic are really affected with an attenuated crinkle. Thus there appears to be some evidence that *quantity* of dose in the mosaic group of viruses governs to some extent the symptoms and degree of virulence of the consequent disease, although this seems inconsistent with some of the writer's earlier experiments with the virulent virus on tobacco produced by progressive inoculations. In these experiments an infinitesimal quantity of virus produced a disease similar in all respects to that produced by a heavy dose of the same virus. The third point emphasised in these studies is the difference in varietal reaction exhibited by potato plants to the same virus quite apart from any transmission through tobacco. It has been seen that the reactions of Great Scot and Kerr's Pink to the crinkle virus were to produce crinkle and streak lesions, and those of President and Arran Victory to produce crinkle only. This can be interpreted in two ways, either to look upon the streak in Great Scot and Kerr's Pink as the normal presentation of this particular crinkle in these varieties, or to regard Kerr's Pink and Great Scot as susceptible to the streak, and President and Arran Victory as "carriers." In this last connection, careful observation has shown that in President the streak element was always rather near the surface, so to speak. For example, one plant, grafted with a scion of Myatt's Ashleaf crinkle and which had developed the normal crinkle, showed numbers of streak lesions, some 3 months after the appearance of the crinkle, and when the plant was fully mature. Probably President infected with Myatt's Ashleaf crinkle and grown under adverse conditions might develop streak without the previous passage of the virus through tobacco. In the same way interveinal mosaic from President produced streak as well as interveinal mosaic when the virus was transmitted to Arran Victory and Great Scot, but interveinal mosaic only on healthy President, unless the virus had been previously passed through tobacco, when a virulent streak developed on President also.

It will be evident from these experiments how important a part streak plays in studies on potato virus diseases, and how urgent a matter it is that its nature and exact connection with the mosaic group should be determined.

8. Summary.

1. Experiments are described on the transmission by different methods of two potato viruses, crinkle on Myatt's Ashleaf potato, and interveinal mosaic on President potato.

2. The virus of potato crinkle was transmitted by needle scratch to President, Arran Victory and Great Scot potatoes. President developed a mosaic mottling, Arran Victory a crinkle similar to that shown by the source of infection, while Great Scot developed crinkle, together with numbers of streak lesions on the leaves. Negative results were obtained with Big Ben and Up-to-Date.

3. Attempted transmission of the crinkle virus by means of the aphis *M. persicae* to potato varieties, President, Arran Victory, Great Scot and Kerr's Pink, was successful only with President, which developed a crinkle of a mild type.

4. Crinkle scions were grafted on to healthy President, Arran Victory, Great Scot and Kerr's Pink. The first two developed crinkle in a form comparable to the source of infection; Great Scot developed a severe streak which attacked the growing points; there was little or no crinkling; Kerr's Pink showed streak lesions accompanied by mottling.

5. Needle inoculation of tobacco with the crinkle virus produced a severe necrotic disease, which increased in virulence by progressive needle inoculation through tobacco. When this virus was returned to healthy potatoes of nine different varieties, including Up-to-Date which is capable of "carrying" streak, all nine varieties developed a severe crinkle and leaf-drop streak. Big Ben was the most susceptible and was killed in very case.

6. Aphis (*M. persicae*) inoculation of tobacco with the same crinkle virus produced different symptoms from those of the needle; these consisted of spots and faint green lines. When this virus was returned to healthy potato by aphis or needle, crinkle and leaf-drop streak resulted.

7. Needle inoculation of *Datura stramonium* with the crinkle virus produced a well-marked mottling of light and darker green; when this disease was returned by needle to President potato, a mosaic mottling only resulted.

8. The virus of interveinal mosaic on President potato was transmitted by *M. persicae* to healthy President; negative results were obtained with Arran Victory.

9. The virus of interveinal mosaic was found to be transmissible by the needle to healthy President.

10. Interveinal mosaic was transmitted by grafting to President, Arran Victory and Great Scot. President showed interveinal mosaic only. Arran Victory showed interveinal mosaic together with numerous streak lesions, while Great Scot developed a form of leaf-drop streak.

11. The virus of interveinal mosaic, when transmitted to tobacco by the needle, produced a well-marked ringspot. When this was returned to potato by needle, interveinal mosaic developed, with the addition of streak lesions and marked curling and distortion of the leaves. Progressive inoculation through tobacco of the ringspot induced by interveinal mosaic gave an increased virulence. When this virulent virus was returned to healthy potato by needle, a severe form of streak resulted, which obscured the symptoms of interveinal mosaic.

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EXPLANATION OF PLATES XVII—XX

PLATE XVII.

Fig. 1. Leaves of potato, Myatt's Ashleaf, showing crinkle; this was the source of infection used in the crinkle experiments.

Fig. 2. Tobacco, White Burley, inoculated by means of the aphis (*Myzus persicae*) from crinkled Myatt's Ashleaf. Note the faint lines and spots of darker green; compare Fig. 3.

Fig. 3. Tobacco, White Burley, inoculated by the needle from crinkled Myatt's Ashleaf. Compare Fig. 2.

Fig. 4. Tobacco, White Burley, showing the increase in virulence of the virus, induced by progressive needle inoculation. This is the fifth plant in the progressive series, the virus being taken from the plant shown in Fig. 3.

PLATE XVIII.

Fig. 1. Potato, Big Ben, killed by a virulent streak induced by needle inoculation from the tobacco plant shown in Plate XVII, fig. 4. The potato illustrated was an exceptionally strong and well-grown plant prior to inoculation; symptoms developed 8 days after inoculation and the plant died with great rapidity.

Fig. 2. Potato, Big Ben, needle-inoculated with the crinkle virus from tobacco. This illustrates the progressive nature of the leaf-drop streak; beginning with the lowest leaves the disease passes rapidly up the plant until the topmost shoot is destroyed. The leaves remain attached to the plant in the manner shown.

Fig. 3. Potato, President, needle-inoculated with the crinkle virus from tobacco. The same leaf-drop streak of a progressive nature develops, but in this variety usually

stops short of the topmost shoot which shows a marked crinkle, but is not killed. Note that the inoculated leaf in the lower right-hand corner is not diseased.

Fig. 4. Potato, President, aphis-inoculated (*M. persicae*) with the crinkle virus from tobacco. The same leaf-drop streak developed as with the needle (Fig. 3) but appeared to be slower in its development.

PLATE XIX.

Fig. 1. Potato, Up-to-Date, killed by leaf-drop streak resulting from needle-inoculation with the crinkle virus from tobacco. This potato variety, which is a well-known streak "carrier," gave no reaction when inoculated with the same virus before passage of tobacco.

Fig. 2. Leaf of potato, Big Ben, showing the development of local symptoms on the inoculated leaf 8 days after inoculation with the crinkle virus from tobacco.

Fig. 3. Leaf of President potato, inoculated with the crinkle virus from tobacco, showing streaking of the veins.

Fig. 4. Leaves of President potato, inoculated with the crinkle virus from tobacco, showing the round streak lesions and destruction of the leaf tissue.

PLATE XX.

Fig. 1. Potato, Great Scot, grafted with a scion of crinkled Myatt's Ashleaf showing the type of streak which developed in this variety. Round streak lesions appeared in the leaves, and the growing points were killed.

Fig. 2. Leaf of *Datura stramonium*, needle-inoculated with the virus from crinkled Myatt's Ashleaf, showing the type of mottling which developed.

Fig. 3. Leaf of President potato, needle-inoculated with the virus of interveinal mosaic which had been passed once through tobacco. Note the development of round streak lesions and that the disease has retained its interveinal character.

Photographs by C. W. Williamson.

(*Received October* 17*th*, 1929.)

Fig. 1.

Fig. 2.

Fig. 3.

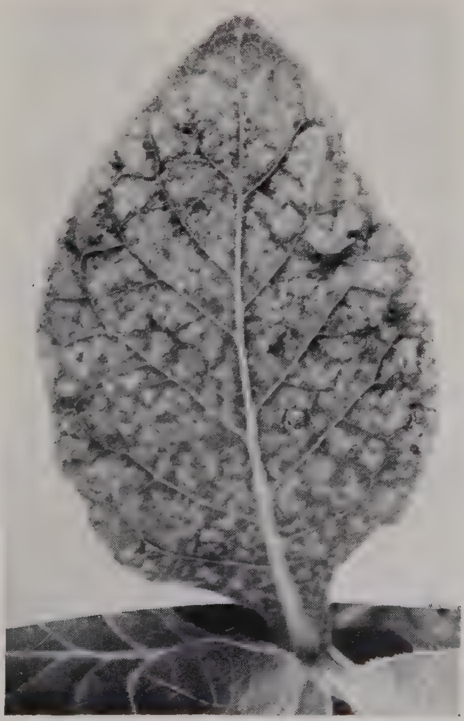

Fig. 4.

KENNETH M. SMITH.—STUDIES ON POTATO VIRUS DISEASES (pp. 223–240).

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

KENNETH M. SMITH.—STUDIES ON POTATO VIRUS DISEASES (pp. 223–240).

Fig. 1.

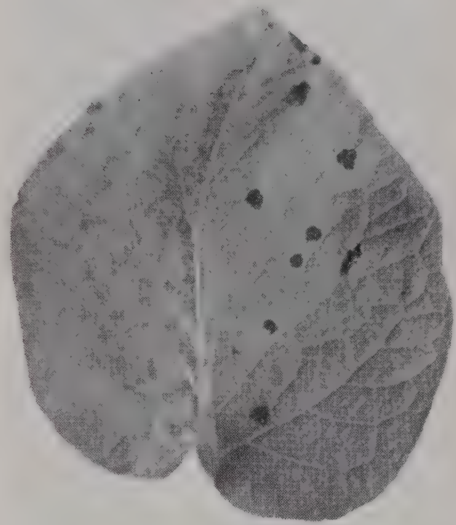

Fig. 2.

Fig. 3.

Fig. 4.

KENNETH M. SMITH.—STUDIES ON POTATO VIRUS DISEASES (pp. 223–240).

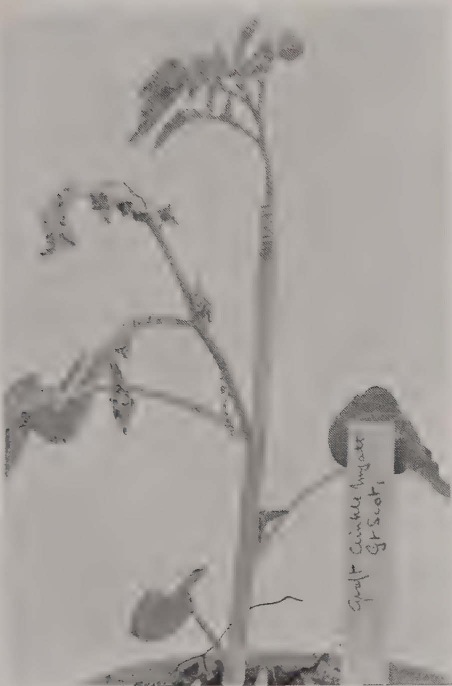

Fig. 1.

Fig. 2.

Fig. 3.

KENNETH M. SMITH.—Studies on Potato Virus Diseases (pp. 223–240).

THE CHLOROTIC DISEASE OF THE HOP

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(With Plates XXI and XXII.)

Historical and Descriptive.

In June 1927 we received from a hop-grower, farming near Tenbury, Worcestershire, a hop plant of the variety Fuggles affected with a disease which was new to us. The grower wrote: "You will notice that the leaves are turned yellow. This has happened to the same plants on the same patch of ground every year for the last four or five years. The area affected is about one-eighth of an acre. The plants go like this about this time each year. Then they seem to recover and at picking time you can see little or no difference between them and the other plants, except that they are weaker. The disease starts with three or four plants and gradually spreads—but does not seem to spread any further now than it did three years ago. I have seen no signs of it anywhere else in my hop grounds. The soil round this patch is rather more sandy than the rest of the hop yard but the sand extends further than the disease. This year I gave the ground some potash, thinking it might have prevented it, but it has had no effect."

In September 1927 the grower sent two stems (bines) of a plant from the diseased patch and wrote: "These bines in June were in exactly the same state as those I then sent to you. You will notice how they have recovered their colour, making fresh shoots and producing quite a few nice hops at the top of the string. Of course they are a good deal weaker than the rest of the hop yard. I propose to grub the whole of the affected patch and replant it. The disease does not seem to spread at all rapidly but certainly a few more stocks are infected this year than there were last year."

The plants sent to us in June 1927 consisted of five stems (bines) 10 ft. to 12 ft. high, of medium thickness. The growing tips of the bines presented no unusual appearance, but all the leaves, from the lowest upwards, showed a puckered growth which was most commonly in the basal two-thirds of the lamina, the apical one-third being sometimes unaffected. The puckered part was yellow or chlorotic. On some of the leaves the upper surface had become domed to such an extent as to

form an inverted keel at the tip of the leaf. The margins of the leaves were imperfect and irregular.

During the winter of 1927–8 all the stocks that were affected—21 in number—were grubbed, and fresh plants of the Fuggles variety were replanted in their places in the following spring. In May 1929 the grower wrote, regarding the latter: "...now when the hops are getting up the strings, nearly all the roots which we planted last year in the place of those grubbed up are affected by the same variegated disease. This would seem to show that the disease has remained in the ground." These plants were also grubbed up, and in a letter written in October 1929 the grower stated that it was his intention to plant up again to ascertain whether the fallow through the summer had cleared the ground.

During the summer of 1929 an extension of the affected area on the same farm was noticed and in July the grower wrote: "I have marked about 200 stocks which have the variegated disease. They are mostly in Fuggles, a few in Mathons, none in Bramlings. They are generally found two or three near each other. I propose to grub them all this autumn."

The grower has kindly supplied the following information as to the source of the first-affected Fuggles and as to the effect of the disease, whether fatal or not. The Fuggles hops were planted in the year 1918, the roots having been bought from a grower at Wateringbury, Kent. "I think it was in 1924 that I first noticed something wrong with them. The bine of the variegated stocks is very weak and rarely gets more than two-thirds up the strings; sometimes it will make a sort of fresh shoot and bear a few hops but no quantity or size to be worth picking. I do not think the cones are distorted in shape but they are always very small. I think, if left, the disease would kill the hill. I have seen them so nearly gone as to be in my opinion past recovery but under these circumstances have always grubbed them. I have not seen the disease in any other locality."

In October 1927 the grower sent roots of affected plants to Wye and these have provided the material for the experiments described later. The following description of the disease is based on observations made on the same plants when growing in pots or in the Experimental Hop Garden at Wye.

In a plant which has already made growth and has started to climb, some or all of the primary leaves exhibit on the lamina areas of pale yellow or pale greenish yellow. When the leaf is viewed against the light of the sky the colour of the affected parts is primrose yellow. On an

otherwise normal leaf the chlorosis appears most commonly near the extreme edge of the basal lobes of the cordate lamina, but in a leaf which is more diseased the yellow colour extends in a narrow band close beside the veins, sometimes widening to eliminate any intermediate strip of green, and so forming a completely yellow area between the veins. The abnormal coloration may cover only a small part of the lamina or it may be intermixed with the ordinary green colour in an almost equal proportion; again it may invade practically the whole lamina, leaving only the extreme tip of the lobes with healthy colour. The extent of attack seems to be determined at an early stage of development of the leaf, and instances have not been observed in which the chlorosis has spread in a fully expanded lamina.

When the yellow colour occurs near the margin of the leaf, it is commonly associated with excessive serration or with complete absence of serration. Occasionally the serrations remain green and then turn upwards in the form of a fringe. Leaves having large chlorotic areas are very commonly distorted, the green parts continuing growth and developing into domed or bulbous parts and those affected with chlorosis remaining only partly expanded and restricting the even development of the entire lamina. Lack of growth at the chlorotic margin serves to increase the distortion, and parti-coloured leaves, closely down-curled, are a frequent, though not constant, feature of the disease.

In the few examples grown in the open at Wye, the bine was thin and weak but was nevertheless able to produce a small crop of hops. Lateral shoots, which develop in the normal way in the axils of the primary leaves, may bear healthy or diseased leaves. The fact that the primary leaves on the higher part of the bine, together with the leaves of the laterals, are often healthy accounts for the impression mentioned by the grower that the plant is able to "grow out" of the disease. In the case of ten affected plants grown in pots in a cold glasshouse during 1928, the bines were very vigorous and strong. After lateral shoots had grown out, the primary leaves, which were nearly all chlorotic, matured early and fell off. The foliage of all the laterals in these ten cases was healthy.

Microscopical examination of hand sections of chlorotic leaves shows a great reduction in the number of chloroplasts in the cells of the yellow parts of the lamina. These parts are also thinner[1] than the green parts

[1] The transverse section of the leaf measured 72μ–85μ in the chlorotic areas, and 150μ–200μ in the healthy. Palisade cells in the chlorotic areas were only 24μ long as compared with 60μ in the healthy parts of the same leaf.

on account of there being fewer cell layers (five cells as compared with seven) and smaller cells.

Experimental.

Investigations were carried out at Wye during 1928 and 1929 on the transmission of the disease.

1928. (*a*) *Budding.*

On April 15th a bud was taken from a chlorotic plant and inserted into the stem of a pot-plant of the variety Fuggles (Ref. no. Ingleby C., W. 75). The plant used as stock had only one bine which was fasciated near the tip. A length of 20 cm. was cut off, leaving the stem 38 cm. long with five nodes, the cut being made just above the fifth node. At this node one leaf and the bud in its axil was removed by cutting away a shallow strip of the stem about an inch long. The diameter of the internode was only 4 mm. A piece of stem bearing a chlorotic leaf with a bud in its axil was cut to fit the wound made and was bound in place by indiarubber tape. On May 5th the inserted bud had formed a shoot 1 inch long and the opposite bud had developed into a lateral $27\frac{1}{2}$ inches long. At the 3rd and 4th nodes the laterals were removed in order to encourage the growth of those at the node above. At the 2nd node from the ground, however, the laterals were allowed to grow.

No further increase in length was made by the inserted shoot and no chlorosis appeared in its leaves or in those of the stock plant.

In 1929 the plant of Fuggles which had been budded showed unmistakable signs of the disease. On April 16th a dwarf shoot only 1 inch high, with two leaves, showed symptoms of chlorosis; a longer stem (about 30 inches high) on the same plant was healthy. On April 27th the long stem began to show symptoms; at that date it was 33 inches high with seven pairs of leaves. Of these the three upper pairs were yellow-streaked. Another short stem, 8 inches high, had four pairs of healthy leaves. On May 10th the dwarf shoot suddenly shrivelled and died when it had reached a length of $1\frac{1}{4}$ inches.

On May 16th the two stems of this plant measured 7 ft. 3 in. and 3 ft. respectively. On the longer there were fifteen nodes, the leaves of the lowest four of which were unaffected; at the 5th, 6th and 7th nodes the laminae were streaked with yellow and contorted; at the 8th (Plate XXI, fig. 1), the basal lobes and centre of the middle lobe of both leaves were yellow and the laminae were contorted. At the 9th

(Plate XXI, fig. 2) the basal lobes were reduced and the laminae were much curved and yellow. At the 10th (Plate XXII, fig. 1) both leaves were smaller and showed similar symptoms. At the 11th (Plate XXII, fig. 2) the leaves were small and much affected. They differed from the other diseased leaves in that the growth of the healthy parts of the lamina induced a concavity to appear in the dorsal surface in place of the more usual convexity or dome (Plate XXI, figs. 1, 2). At the 12th, 13th and 14th nodes the leaves were all similarly affected; at the 15th they were only just projecting from the covering stipules. On the second stem there were eleven nodes, of which the lowest four showed no disease symptoms. At the 5th to the 8th, chlorosis appeared in varying degree; at the 9th, 10th and 11th the laminae were young and unaffected. The primary leaves on both the bines matured early and dropped off, the chlorotic areas turning brown before the green areas. Laterals up to 2 ft. 6 in. in length grew out and some of their leaves showed signs of the disease. Both stems were able to bear a few small cones.

1928. (*b*) *Grafting.*

Ten chlorotic plants in large pots grown from roots supplied in the previous year were grafted[1] with fourteen scions of the varieties Fuggles (4), Rodmersham Golding (9) and Tutsham (1). With one exception (a scion of Rodmersham Golding which died) all the scions grew strongly and remained healthy throughout the season.

1929. (*a*) *Grafting.*

1. Two scions of the variety Fuggles were grafted in 1929 on each of three chlorotic plants which had been planted out in the Experimental Hop Garden at Wye, two (Ref. nos. V. 96, V. 97) in November 1927 and one (Ref. no. AA. 4) in November 1928. Of the first two scions[2], one was destroyed by accident and the other grew to a height of 6 feet without showing any disease and bore a few normal hop cones. Of the second pair of scions[3], one died and the other reached a height of 6 feet and remained healthy, bearing a few small cones. Of the third pair[4], one scion grew to $4\frac{1}{2}$ feet in height and remained healthy, but the other, grafted on May 13th, showed chlorosis on several leaves on

[1] The method of grafting employed was that described in this JOURNAL, XVI, 359, 1929.

[2] The scions (Ref. no. R. 3, h. 4) were obtained from a plant growing in a commercial hop garden near Wye; the stock was Ref. no. AA. 4.

[3] Ref. no. R. 1, h. 2, from the same commercial garden; on stock Ref. no. V 96.

[4] Ref. no. R. 1/44 *a* from the Experimental Hop Garden, Wye, on stock Ref. no. V. 97.

July 29th when at a height of 5 ft. 6 in. with eighteen nodes. It never grew more than this. The 8th to 15th nodes bore leaves all having symptoms varying from the least to the most chlorotic, accompanied by contortion. At the 16th, 17th and 18th nodes the leaves were small and abnormal with large stipules. Laterals up to 4 inches long developed from six of the nodes and all showed some symptoms of the disease. By September 24th, the healthy scion had produced a few cones but the inflorescences of the infected scion remained as hard, knob-like organs and failed to develop further. All three plants used as stocks in the above experiments showed the disease, but were all able to produce hops on their rather weak bines which reached from 10 to 12 feet in height.

2. The reverse method of grafting was carried out on May 3rd, 1929, when six scions obtained from chlorotic plants were grafted on four healthy plants of the variety Fuggles. The scions, with one exception which died after 2 months, grew and persisted throughout the season. They reached heights varying from 7 inches to $4\frac{1}{2}$ feet and, though some of the lower leaves showed evident chlorosis, the disease did not continue to become apparent in the later-produced leaves higher up the stems.

No chlorosis was transmitted to the stock plants during 1929 by this grafting and they, together with those which were budded (see (*b*) below), will be kept under observation during 1930.

1929. (*b*) *Budding.*

Fourteen chlorotic buds were inserted into the bines of fourteen healthy plants of the varieties Mid-European Golding (7), Fuggles (6), and a New Seedling Hop plant (Ref. no. OK. 50) (1), growing in the Experimental Hop Garden at Wye. No positive results were obtained during the season of 1929.

1929. (*c*) *Rubbing with juice from crushed leaves.*

Chlorotic leaves were crushed in a mortar and the juice was used to rub on the surface of two leaves on each of four healthy plants of the variety Mid-European Golding growing in the Experimental Garden at Wye. No positive results were obtained during the season of 1929.

Fig. 1.

Fig. 2.

SALMON & WARE.—The Chlorotic Disease of the Hop (pp. 241–247).

Fig. 1.

Fig. 2.

SALMON & WARE.—The Chlorotic Disease of the Hop (pp. 241–247).

GENERAL CONSIDERATIONS.

The disease described above evidently belongs to the virus class. It would appear that three virus diseases of the hop exist in this country. The chlorotic disease resembles in some features the nettlehead disease[1] (which attacks the Fuggles variety) both because apparently it spreads from plant to plant only slowly, and also because it does not kill the plant, at any rate for some seasons, but allows it to grow weak bines on which cones are produced. The mosaic disease[2], on the other hand, spreads very rapidly, usually kills the plant in one or two seasons, and is not found in the variety Fuggles.

SUMMARY.

1. A description is given of a new virus disease of the hop characterised by weakness of growth and by the presence of yellowish (chlorotic) markings on the leaves, accompanied by distortion of the lamina. The name "chlorotic disease" is proposed.

2. Two instances are recorded where the disease has been transmitted artificially, in one to a healthy plant by budding with a diseased bud, and in the other to a healthy scion by grafting it on to a diseased stock.

EXPLANATION OF PLATES XXI AND XXII

PLATE XXI.

Fig. 1. The chlorotic disease of the hop. The light coloured parts of the leaf are lacking in chloroplasts. Chlorosis at the margins has caused restriction of growth and consequent curvature of the lamina. Occasionally, as shown here, the distortion takes the shape of a narrow, inverted keel. The photograph, taken on May 16th, 1929, shows the pair of leaves at the 8th node from the ground on a bine of a hop plant (variety Fuggles) which was budded one year previously, when healthy, with a single leaf and bud taken from a chlorotic plant of the same variety. (Nat. size.)

Fig. 2. The leaves at the next node above on the same plant (node 9). (Nat. size.)

PLATE XXII.

Figs. 1 and 2. The leaves at the next nodes above on the same plant (nodes 10 and 11). (Nat. size.)

[1] Grafting experiments carried out in 1929 have shown that Nettlehead is transmissible. Details of the work will be published later.

[2] The literature concerning mosaic disease has been quoted in this JOURNAL, XVI, 381 (1929).

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STREAK—A VIRUS DISEASE OF TOMATOES

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(With 2 Diagrams in the Text.)

Introduction.

Streak disease of tomatoes has for many years been recognised as one of the most severe diseases to which this plant is susceptible. The literature contains, under various names, lengthy descriptions of symptoms of diseases apparently identical with streak. The terms stripe, black stripe, winter blight, severe mosaic, and probably spotted wilt, all appear to have reference to this same disease.

Tomato streak occurs frequently in commercial glasshouses in the south of England, where tomatoes are forced for an early market. It agrees in its symptoms with the description given by Vanterpool (13), Gardner and Kendrick (7), Brittlebank (5), and others, and is probably identical with tomato stripe (Bewley (2)). Many tomato plants attacked by this disease show leaf mosaic symptoms only; others show, in addition to mosaic, large necrotic areas on the leaves accompanied by longitudinal, dark, necrotic lesions on the stems and petioles, whilst on other plants similar necrotic areas may be found without any mosaic being present. Frequently, however, a plant, which at first shows mosaic only or necrosis only, subsequently develops the other type of symptom also.

The evidence for the statement that this disease is either bacterial or malnutritional in origin is inconclusive, and it is now generally recognised to be a virus disease. However, there seems to be little agreement among investigators as to the identity of the virus causing streak. No less than three theories are supported: (*a*) that this disease is due to the virus of tomato or tobacco mosaic—tobacco virus I (Johnson (11)), which under certain environmental conditions takes on increased virulence and causes necrosis in addition to mosaic; (*b*) that two components are concerned in the virus of streak, one producing the mosaic, and the other, the necrotic streaks (cf. Boning (4)); (*c*) that streak is the result of a mixed infection by the viruses of tobacco mosaic, and potato mosaic

(cf. Dickson (6) and Vanterpool (13)). The disease resulting from the combination of the two latter viruses is admittedly indistinguishable from glasshouse streak, and is termed "experimental streak" in the work to be described.

Blood (3) claims to have produced a streak disease in tomatoes by inoculation with "a disturbing principle from apparently healthy potatoes in combination with tomato mosaic virus." However, in view of the failure of Henderson Smith (9) in tomato and of Kenneth Smith (12) in tobacco to produce any disease by inocula from absolutely healthy potatoes, it is probable that the potatoes employed by Blood contained a mosaic which was masked.

Berkeley (1), on the other hand, states that "it is not necessary to have a combination of viruses in order to produce streak, since the juice of healthy potatoes in itself is sufficient for this purpose." However, in this conclusion, this worker appears to have confused with streak the disease which is produced in tomatoes by the juice of apparently healthy potatoes (Johnson (10)) or of potatoes infected with mosaic (Henderson Smith (9)).

EXPERIMENTAL.

This paper is concerned with a comparison of glasshouse streak and experimental streak. The source of glasshouse streak was a commercial glasshouse in which the incidence and spread of the disease among tomato plants was watched during several months. Separate samples were taken from plants showing the three forms of the disease, viz. those showing mosaic only, those showing necrosis only, and those showing mosaic and necrosis on the same plant. The virus of tobacco mosaic tobacco virus I (11) was furnished by Dr Grainger of Leeds University, who obtained it from Dr Johnson of Wisconsin. The virus of potato mosaic was derived by Dr Henderson Smith from mosaic Up-to-Date potatoes, the leaves of which when inoculated into tomato produced a characteristic disease (8).

METHODS.

Preparation of plant extracts. In all cases this was done by grinding the minced leaves of diseased plants with water in a mortar, 3 c.c. of water being added for each gram of leaf tissue. For immediate transmission of a disease from one series of plants to another, inoculation was made with this pulp, but when a stock of sterile infective juice was required, the method of filtration described by Henderson Smith (8) was

adopted. The filter cylinder used preparatory to the Pasteur Chamberland filters was composed of alternate layers of sand and macerated ashless filter paper tightly packed. The final dilution of plant juice in water was not greater than 1 in 9 in the case of tomato, and 1 in 5 or 6 for tobacco extracts. To prevent evaporation, paraffin wax was added to the cotton wool plugs of the tubes containing the extracts, and in this way a bacteriologically sterile stock can be kept indefinitely.

Methods of inoculation. Inoculations were usually made by pricking about forty times with a needle, three or four of the youngest leaves of a young vigorously growing, healthy plant. For this purpose the leaf was placed in the inoculum lying on the flat wooden label to be used for that particular plant. In some cases, the plants were inoculated at the base of the stem below the first leaves, by three or four longitudinal incisions in the succulent stem, and into these incisions the extract was inserted. Both methods gave positive results with equal regularity, although the incubation period following inoculation by the second method was invariably at least one day longer.

When individual plants were inoculated with two viruses, as, for example, in the production of experimental streak, either equal volumes of the extracts containing each different virus were mixed together and used as one, or both extracts were inoculated separately into two leaves. These methods were equally effective.

Precautions were taken to protect the experimental plants from secondary infection.

Details of experiments.

The first experiment was carried out with the original diseased tomato plants from the commercial glasshouse. From the difference in appearance of the two types of symptoms (the coarse mottle or mosaic and the necrosis of the leaves and stems) streak, as has been mentioned above, has been regarded as being due to two factors. This experiment was designed to show whether this distinction of symptoms remained constant, and to ascertain the effect of filtration on the infectivity of the inoculum. To this end, filtered and unfiltered extracts of plants showing each type of symptom independently, and of plants showing both forms together were inoculated into tomato plants (variety Kondine Red) and tobacco plants (variety White Burley), seven plants being used in each series. As a control, the original plants (two in each case) were grown in the experimental glasshouse, and in addition two cuttings were made from each of them. One of the two plants showing mosaic

only developed in course of time necrotic spots on the upper leaves and typical lesions on the stem, while the leaves from both of its cuttings developed large necrotic areas. Both the cuttings and also the new apical and lateral shoots on both plants formerly showing streak only developed conspicuous mosaic symptoms.

Table I.

Inoculum		Tomato. Symptoms produced: Mosaic only	Tomato. Symptoms produced: Mosaic and streak	Tobacco. Symptoms produced: Mosaic only	Tobacco. Symptoms produced: Mosaic and streak
Mosaic only:	Filtered	5	2	2	5 (1 killed)
	Unfiltered	6	1	2	5
Streak only:	Filtered	7	0	3	4 (1 killed)
	Unfiltered	5	2	2	5 (2 killed)
Mosaic and streak:	Filtered	6	1	3	4 (1 killed)
	Unfiltered	4	3	4	3

The results given in Table I show that, judged by the symptoms produced on sub-inoculation, no difference could be detected between the different inocula. Glasshouse streak, no matter how virulent it appeared to be in the inoculum, did not produce pure streak (*i.e.* necrotic) symptoms when inoculated into healthy plants, nor does pure mosaic in the inoculum mean that streak may not result on sub-inoculation. Moreover, filtration through Pasteur Chamberland L. 1 and L. 3 candles does not reduce the infectivity of the disease.

Resistance to heat and alcohol. It was thought that the mosaic and the streak factors, if these be distinct, might be differentiated by their reactions to various degrees of heat and alcohol. Accordingly, filtered extracts from single tomato plants showing both coarse mosaic and streak on the one plant were (1) heated for 10 minutes at 60°, 70°, 80°, 85° and 90° C. and immediately afterwards cooled under running water, and (2) treated for 1 hour at concentrations of 60, 70, 80 and 90 per cent. alcohol. The precipitate formed by the action of the alcohol on the plant juice was separated from the supernatant liquid by centrifuging, and then shaken up in distilled water, making the final volume up to the original volume of plant juice. Subsequent inoculations showed that the precipitate contained the virus, and the supernatant liquid did not.

The virus was inactivated by 90° C. for 10 minutes, but retained its virulence at 85° C., and also after treatment with 90 per cent. alcohol for 1 hour. This experiment was repeated with extract from tobacco inoculated with glasshouse streak with essentially the same results.

Although the proportion of streaked plants was low, mosaic and streak symptoms appeared with equal regularity over the same range of heat and alcohol concentration.

Table II.

(*a*) *Effect of heat on the infectivity of glasshouse streak.*

Inoculum	10 mins. at	No. of plants	No. positive	Symptoms
Glasshouse streak showing both mosaic and streak	60° C.	7	7	6 mosaic only, 1 mosaic and streak
	70	7	7	4 ,, 3 ,,
	80	7	7	5 ,, 2 ,,
	85	7	7	5 ,, 2 ,,
	90	7	0	Nil

(*b*) *Effect of alcohol on the infectivity of glasshouse streak.*

Inoculum	Alcohol strength %	No. of plants	No. positive	Symptoms
Glasshouse streak showing both mosaic and streak	60	7	7	5 mosaic only, 2 mosaic and streak
	70	7	7	4 ,, 3 ,,
	80	7	7	5 ,, 2 ,,
	90	7	7	6 ,, 1 ,,
Control untreated	—	7	7	5 ,, 2 ,,

Excessive nitrogen manuring and forcing of young plants is reported by growers and others to induce the formation of streak, and these treatments were tried on the plants used in the above experiment. At the end of the fourth week excessive nitrogen (1·13 gm. of sodium nitrate per 6-inch pot) was added to three plants in each series, while the tops of the other three were forced by cutting away all axillary buds and shoots. However, only four more plants out of sixty so treated developed streak after this date, and they are not included in the table above as they developed it after so long an interval that secondary infection could not certainly be excluded.

In all experimental plants showing mosaic only, the close similarity of the symptoms to those of tobacco mosaic in tomato was particularly noticeable, and the comparison may be extended when one examines the resistance of tobacco virus I to heat and alcohol (see Table III). It will be seen that this virus retains its activity entirely after treatment with 90 per cent. alcohol and almost entirely after heating for 10 minutes at 85° C., results which agree with those of others. Tobacco mosaic, therefore, shows resistance of the same order as that of glasshouse streak to alcohol, and heat.

Table III.

(*a*) *Effect of heat on the infectivity of tobacco virus I.*

10 mins. at	No. of plants	No. positive	Symptoms
60° C.	7	7	Mosaic only
70	7	7	,,
80	7	7	,,
85	7	6	,,
90	7	0	Nil

(*b*) *Effect of alcohol on the infectivity of tobacco virus I.*

1 hour of	No. of plants	No. positive	Symptoms
70 %	7	7	Mosaic only
80	7	7	,,
90	7	7	,,
Control untreated	7	7	,,

Combined inoculations. From the above comparisons it seemed probable that glasshouse streak would act in the same way as tobacco mosaic when combined with potato mosaic—that is, produce experimental streak. Potato mosaic was accordingly combined with tobacco mosaic in one series of inoculations, the combination producing a disease here referred to as experimental streak 1; and in another series it was combined with glasshouse streak, and produced a disease, here called experimental streak 2. These forms of streak were identical in the length of incubation period, as well as in the manner of appearance and intensity of symptoms. On the other hand, the combination of tobacco mosaic and glasshouse streak produced the symptoms of tobacco mosaic only, until on the 22nd day one of the seven inoculated plants developed necrotic leaf spots without stem lesions—*i.e.* this combination did not produce streak.

Resistance to ageing in vitro. Filtered extracts of glasshouse streak, tobacco mosaic, potato mosaic and experimental streaks 1 and 2 were kept bacteriologically sterile in sealed test tubes in a cupboard, in subdued light, and after varying lengths of time their virulence was tested by inoculation into healthy tomatoes.

From Table IV it is seen that the virus of glasshouse streak in tomato or tobacco extract retains its virulence stored *in vitro* for at least 16 months, while tobacco virus I in tomato extract is virulent after 12 months under similar conditions. Dickson (6) records that expressed juice of mosaic diseased tobacco plants, unfiltered and protected from contamination by a layer of toluene, was still virulent after storing for 5 years. On the other hand, potato mosaic appears to have lost its

Table IV.

(*a*) *Single viruses.*

Virus	Age in months	No. of plants	No. positive	Symptoms	
Glasshouse streak in tomato extract	6	7	7	*7 mosaic only	
	9	6	6	5 „	1 mosaic and streak
	12	6	6	4 „	2 „
	16	6	6	*6 „	
Glasshouse streak in tobacco extract	6	7	7	*7 „	
	9	6	6	3 „	3 „
	12	6	6	2 „	4 „
	16	6	6	*6 „	
Tobacco mosaic in tomato extract	6	6	6	6 mosaic	
	12	6	6	6 „	
Potato mosaic in tomato extract	3	6	6	6 fine spot necrosis	
	5	6	6	6 „	
	6	6	0	Nil	
	9	6	0	Nil	
Potato mosaic in tobacco extract	9	6	0	Nil	

* In each of these cases, the juice was tested in late autumn when growth of the plants is very slow and glasshouse streak symptoms are not usually obtained in England.

(*b*) *Viruses in combination.*

Virus	Age in months	No. of plants	No. positive	Symptoms	
Experimental streak 1 in tomato extract	3	7	7	7 experimental streak	
	5	7	7	7 „	
	6	6	6	6 mosaic only	
	12	6	6	6 „	
Experimental streak 2 in tomato extract	5	7	7	6 experimental streak,	1 mosaic only
	6	12	12	1 „	11 „
	8	6	6	6 mosaic only	
	12	6	6	6 „	

power of infection after 6 months' storage *in vitro* in tomato or tobacco extract, since it no longer produces a disease in tomatoes. According to Henderson Smith (9), this mosaic virus from Up-to-Date potatoes was inactive after 12 weeks in filtered tomato juice, while the virus of mosaic in the variety Majestic remains infective for at least $5\frac{1}{2}$ months. Extracts from plants which have been inoculated with two viruses, viz. experimental streak 1 or 2, have power to reproduce these diseases entirely for only 5 to 6 months, after which time it is assumed that the potato virus has lost its virulence, for the resulting symptoms are those of tobacco mosaic, or the mosaic of glasshouse streak only. After 12 months —no longer period has yet been tried—these symptoms appear regularly.

Resistance to ageing *in vitro* is a character which is preserved by individual viruses alone, or when in combination in plant extracts. This property is useful along with resistance to heat and alcohol in separating a single virus from a mixture. It is interesting to note that the virus of potato mosaic is less resistant than tobacco virus I or glasshouse streak to all three of these treatments.

Host range. Use of a number of different hosts as a means of separating and identifying viruses was made with special regard to two characters, the symptoms produced by each virus, and the length of time between inoculation and appearance of these symptoms, that is the incubation period of the virus in the host. The host plants tested were *Nicotiana tabacum* (var. White Burley), *N. affinis*, *Lycopersicum esculentum* (var. Kondine Red), *Solanum nigrum*, *S. dulcamara*, *S. villosum*, *S. nodiflorum*, *Nicandra physaloides*, *Petunia violacea*, *Hyoscyamus niger*, *Datura stramonium* and *Cucumis sativus*. Glasshouse streak and tobacco mosaic were the inocula, six plants being used in each series. All the above-mentioned plants, except *Datura stramonium* and Cucumber, showed pronounced leaf mosaic often with some distortion due to the irregular raised dark green areas, symptoms characteristic of tobacco mosaic. Not only did the same plants take both these viruses but they showed identical symptoms developing after the same incubation period.

Cucumber showed no symptoms at all with either virus, while *Datura stramonium* showed only frequent, dark brown, stem lesions below the nodes of the lower leaves, and on two plants slight necrosis of the leaf at the site of inoculation. Bewley (2), working with mosaic disease of tomato in *Datura stramonium*, says that no mosaic symptoms developed, but that all the plants showed "Stripe," a term which appears to be synonymous with "Streak." It is possible that he referred to these brown stem lesions also.

On the other hand, *Datura stramonium* has been shown to take potato mosaic, giving pronounced leaf symptoms (Henderson Smith (9)). This plant therefore suggested a means of separating potato mosaic from its combination in experimental streak 1 and 2. Also, if glasshouse streak contains this factor, *Datura stramonium* would be expected to show it.

Potato mosaic was transmitted to *Datura* in each case and from *Datura* back to tomato, but this did not occur with glasshouse streak. In experimental streak 1 and 2, the potato mosaic component appeared to be responsible for the leaf symptoms in *Datura*, but the dark stem lesions produced by tobacco mosaic and glasshouse streak were also

formed; only the potato mosaic virus, however, was transmitted back to tomato. It appears from these results that potato mosaic was not present in the glasshouse streak used.

Table V.

(*a*) *Tomato*—Datura *inoculations.*

Host	Inoculum	No. of plants	No. positive	Symptoms
Datura stramonium	Glasshouse streak	8	8	Brown stem lesions; plant No. 2 showed necrosis of veins of an inoculated leaf
	Potato mosaic	8	8	Fine mottle on leaves, fine rings formed and frequent small necrotic spots
	Experimental streak 1	8	8	Fine mottle on leaves, with brown stem lesions in all plants
	Experimental streak 2	8	8	Fine mottle on leaves in all and brown stem lesions in 7 plants

(*b*) Datura—*Tomato inoculations.*

Leaves of plants Nos. 1 and 2 from each of the above series were then inoculated back into tomato, 3 weeks later.

Host	Inoculum	No. of plants	No. positive	Symptoms
Tomato	Glasshouse streak	8	*1	Typical coarse mosaic
	Potato mosaic	8	8	Necrotic leaf spots and fine mottling
	Experimental streak 1	8	8	,, ,,
	Experimental streak 2	8	8	,, ,,

* This plant was inoculated with material which contained the necrosed area of plant No. 2 noted in the corresponding series above; it is probable that in this necrotic area the glasshouse streak of the original inoculum remained virulent and produced the mosaic in this single plant when reinoculated into tomato.

Discussion.

Many workers dealing with streak disease of tomatoes have experienced difficulty in reproducing streak symptoms regularly, by artificial means of inoculation, a difficulty which was not overcome by the writer. On the other hand, experimental streak can invariably be reproduced. In the following diagrams we have this difference shown clearly.

Six plants were inoculated with glasshouse streak (Diagram 1), and six with experimental streak (Diagram 2). In both cases the inoculum had been stored *in vitro* for 6 months and in the latter—experimental

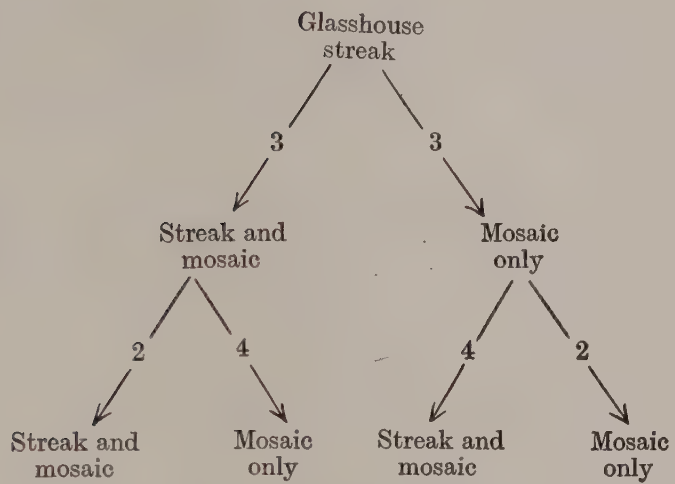

Diagram 1.

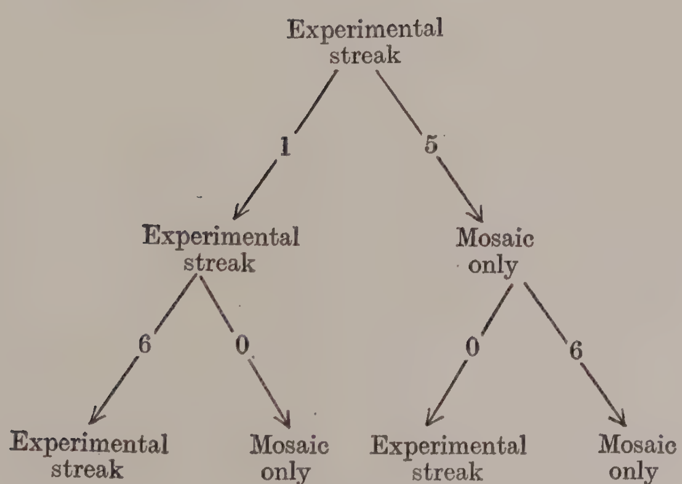

Diagram 2.

streak—the infectivity of the potato mosaic factor was so reduced that only one out of six plants developed experimental streak, but successive sub-inoculations from the other five plants produced the coarse mosaic of tobacco virus I only, as was to be expected. With glasshouse streak, streak and mosaic symptoms appear irrespective of the symptoms shown by the plants from which the inoculum was derived.

In experimental streak, the virus of potato mosaic appears to be the factor responsible for the necrosis of the plant, for tobacco virus I alone rarely, if ever, produces necrosis in tomato. It is difficult to understand why the addition of this potato virus to tobacco virus I should cause such a virulent disease which may practically kill its host.

No indication that glasshouse streak contains the virus of potato mosaic has been found, and necrotic lesions have occurred after juice containing the virus of glasshouse streak has been subjected to treatments with alcohol and heat and storage *in vitro* calculated normally to destroy the infectivity of potato mosaic.

The complete agreement in symptoms, resistance to alcohol, heat and ageing *in vitro*, host range and general characters which has been shown in this paper suggests that the virus of tobacco mosaic and glasshouse streak are probably one and the same. As glasshouse streak has been shown not to contain potato mosaic, necrosis must be due to another factor, perhaps connected with the reaction of the host and its physiological condition at the time of inoculation or, as generally assumed, necrosis only occurs under certain experimental conditions which have still to be defined.

Summary.

A comparison of streak disease of tomatoes, derived from commercial glasshouses, and experimental streak produced by combined inoculation of the viruses of potato mosaic and tobacco mosaic, is given in detail.

The characters employed in comparison are the host range of each virus and its resistance to various temperatures, to different concentrations of alcohol, and to ageing *in vitro*.

Glasshouse streak and tobacco mosaic show an equal resistance to alcohol, heat and ageing *in vitro*, and have, in addition, an identical host range. Treatment for 1 hour with 90 per cent. alcohol and for 10 minutes at 85° C. did not destroy the infectivity of either of these viruses.

Glasshouse streak is shown not to contain the virus of potato mosaic, but is of itself able to produce necrosis in tomatoes without the partici-

pation of potato mosaic. The factors underlying this have not been determined.

It is concluded that tobacco mosaic and the mosaic of glasshouse streak are probably identical, and that much of the streak occurring in glasshouses is due to a single virus, and not a mixed infection of this with potato mosaic.

The author wishes to express her indebtedness to Sir John Russell, F.R.S., Director of the Rothamsted Experimental Station, for putting at her disposal the facilities of the Station, and to Dr W. B. Brierley, Head of the Department of Mycology, in which this work was carried out, the author at the time being the holder of a research studentship awarded by the Australian Council of Scientific and Industrial Research.

The assistance given by Miss M. M. Browne in the growth and care of the many experimental plants required in this work is gratefully acknowledged.

The thanks of the author are especially due to Dr J. Henderson Smith, whose ready advice and assistance throughout have been invaluable.

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THE CONTROL OF CUCUMBER AND TOMATO MOSAIC DISEASES IN GLASSHOUSES BY THE USE OF CLEAN SEED

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SEED transmission of mosaic disease has been demonstrated in a number of vegetable and field crops. In the case of the common bean, *Phaseolus vulgaris*, Reddick and Stewart (9) obtained 50 per cent. of diseased plants from seed taken from infected parents; Gardner and Kendrick (7) found that soybean mosaic was similarly transmitted but to a less extent. Newhall (8) working with lettuce obtained 2–8 per cent. seed transmission, while Dickson (3) reported positive results of "seed inheritance" in the mosaic diseases of several legumes, notably *Trifolium pratense*, *T. hybridum*, *Melilotus alba* and *Pisum sativum*, as well as Hippeastrum species.

Seed transmission is not generally accepted for the cucumber and tomato, for while Westerdijk (10) upheld it on the basis of a small test, Allard (1) and Gardner and Kendrick (6) obtained negative results in spite of extensive experiments.

Similarly Doolittle (4) concluded after repeated experiments with cucumber mosaic that seed transmission does not occur, although in an earlier paper Doolittle and Gilbert (5) published positive evidence of seed transmission in the case of the wild cucumber, *Micrampelis lobata*.

Both cucumber and tomato mosaic diseases take annual toll of commercial crops in this country. The extent of the damage varies considerably from season to season, being affected by certain physical conditions of the plant's environment, which will be discussed in another paper. These diseases have been under observation and investigation by the senior author since 1919, and the information collected together with that supplied by commercial growers in the Lea Valley suggests that these diseases may tend to run in cycles.

In 1919 cucumber mosaic was only observed on 2 nurseries out of 87 visited; in 1920 it was found on 7; and in 1921 on 29 nurseries. During 1922 mosaic disease was widely spread, being found on 72 nurseries. From 1923 to 1925 the disease gradually became less prevalent, and

during 1926, although found on 31 nurseries, it appeared to have lost much of its virulence. In 1928 there were signs that it was increasing in extent and during the present season it is widespread.

Table I.

Prevalence and intensity of cucumber mosaic, 1919–29.

Year	Prevalence	Intensity
1919	+	W.
1920	+	W.
1921	+ + +	S.
1922	+ + + + +	M.
1923	+ +	W.
1924	+ +	S.
1925	+ +	W.
1926	+ +	W.
1927	+ +	W.
1928	+ + +	S.
1929	+ + + + +	S.

W. = weak. M. = moderate. S. = strong.

In the above table an attempt has been made to convey some idea of the prevalence of cucumber mosaic disease and the intensity of its symptoms during the last 10 years. Plus signs are used to illustrate the prevalence of the disease, because figures are not available to express accurately the percentage of infected nurseries in any one centre.

It will be seen that the intensity of the symptoms varies in different years, high intensity not necessarily coinciding with greatest prevalence. Indeed symptoms are usually aggravated by high temperatures such as occurred during 1921, 1924, 1928 and 1929.

Similar observations have been made in relation to the tomato, although the periodicity effect is not so marked.

The above observations suggested that mosaic disease of both the cucumber and the tomato may be transmitted in the seed. Confirmation of this was sought in utilising information from many thousands of plants, by following up the history of different batches of seed which have been saved and grown by nurserymen.

Table II provides information concerning the incidence of cucumber mosaic on commercial nurseries, where different batches of seed were grown in different blocks of houses. It will be seen that while some blocks remained free from the disease others were badly infected. In no case could any reason be: ound for this variation in disease incidence other than seed transmission.

Table II.

The presence of mosaic disease in cucumber plants raised from different batches of seed.

Nursery	Year in which seed was sown	Year in which seed was taken							
		1916	1921	1922	1923	1924	1926	1927	1928
1	1923	H.	—	M.	—	—	—	—	—
2	1924	—	M.	M.	H.	—	—	—	—
3	1924	—	H.	M.	M.	—	—	—	—
4	1924	—	M.	H.	H.	—	—	—	—
5	1925	—	M.	M.	H.	H.	—	—	—
6	1926	—	H.	H.	M.	M.	—	—	—
7	1928	—	—	—	—	—	H.	—	—
8	1928	—	—	—	M.	—	—	H.	—
9	1929	—	—	—	—	—	H.	H.	M.
10	1929	—	—	—	—	—	H.	—	M.
11	1929	—	—	—	—	—	M.	—	M.

H. = healthy. M. = mosaic.

In addition, the appearance of a small percentage of infected plants, both cucumbers and tomatoes, constantly occurs on nurseries as early as 3 weeks from sowing the seed, even when the conditions apparently preclude the possibility of infection from outside sources. The following case is a striking example of this. During 1923 attention was drawn to a nursery where 100,000 tomato seedlings were being prepared for planting. The seed had been sown in sterilised soil in new boxes on January 15th, and potting started on February 14th. Inspection occurred on March 4th, when approximately 80 per cent. of the total plants showed infection by mosaic disease. The propagating houses had been thoroughly cleansed in the winter by spraying the superstructure with emulsified cresylic acid delivered with considerable force from a pressure machine, and watering the soil surface with cresylic acid and water. The staging too, on which the pots rested, had been similarly treated. In spite of careful search no signs of infection by green fly, white fly or the red spider mite could be seen. A mass infection of this extent seemed to offer strong evidence in support of seed transmission.

In view of this evidence an attempt was made to obtain seeds free from mosaic disease, and in 1922 Mr H. O. Larsen very kindly supplied a quantity of Butcher's Disease Resister cucumber seeds, which had been saved in 1916 and stored in a sealed tin since that year. This seed had been taken from plants in a nursery where mosaic disease had not been noticed prior to 1921. One thousand plants were grown in the experimental houses during 1923 and remained free from mosaic throughout the season. Seed was taken and stored for further use. Previous

crops in these houses 1920–2 were badly infected with the disease, which also appeared in 1924 when seed was used from a commercial source.

In July 1924 some of the seed saved from the healthy crop of 1923 was supplied to a grower whose cucumber crop had suffered badly from mosaic disease during the previous five years, and where all attempts to control it had failed. By growing this small batch of seed in a tomato house situated in a district free from cucumbers, sufficient seed was prepared for 5 acres of cucumbers on the infected nursery. This seed was used in 1925, and for the first time in six years the plants were free from the disease except in three small houses. It was found that the man in charge of these houses used to visit, during the evenings, a neighbouring nursery where mosaic disease existed, and probably infection was carried in this way. The houses were isolated, and the rest of the nursery remained clean. Seed was taken from selected plants and no further recurrence of the disease has taken place since 1924. Similar results have been obtained on two other nurseries.

Accurate proof of seed transmission long defied all attempts to secure it by the standard methods, but recently 6·04 per cent. of infected seedlings has been obtained from a batch of 493 tomatoes grown from seeds taken from infected fruits. Some success has also been obtained by inoculating healthy tomato plants with the crushed embryos of seeds taken from copiously infected plants growing in 12-inch pots.

Immature seeds were taken out of the unripe fruit of a plant severely infected with "Aucuba" mosaic. The seeds were first washed in 80 per cent. alcohol for 15 minutes and then in several changes of distilled water. After thoroughly washing, the embryos were dissected from the seeds and washed in several changes of sterile distilled water. An inoculum was prepared by crushing with a little distilled water, and tomato plants were inoculated. The inoculated plants were kept in a chamber of a glasshouse which contained no other mosaic infected plants. Of the six plants inoculated, two showed definite symptoms of mosaic in 21 days and, in 27 days, two others developed symptoms.

The experiment was repeated with two further batches of seed. In these cases the embryos were washed in running water for a period of 24 hours before being crushed and inoculated into tomato plants. From these two batches 24 plants were inoculated, and of these five developed mosaic.

In all these experiments care was taken to prevent external infection by insects and contact with diseased plants, by isolation and regular fumigation.

The plants used for inoculation were taken from a batch of 200. Mosaic disease did not occur in any of the plants except those inoculated.

The importance of clean seed being realised, two glasshouses were erected at Cheshunt for the purpose of cleaning existing stocks of cucumber and tomato seeds. The cost of this work was defrayed by the Empire Marketing Board, who also provided an annual maintenance allowance.

For the purpose of this work suspected stocks of Butcher's Disease Resister cucumber and E.S. 1 tomato seeds were used.

Cucumber selection.

The seeds were sown on December 21st, 1927, in new boxes, using a compost sterilised by heat. They were potted into size "32" pots on January 21st, 1928, and 100 were planted into the special house on February 12th. The first appearance of mosaic occurred on six plants during the first week in March. These plants were immediately uprooted and destroyed, as were any that appeared later. In all, 17 plants out of 100 developed disease symptoms and were removed. From the beginning two female flowers were hand pollinated and two seed fruits were obtained from each plant. The seed was extracted in April, keeping that from each plant separate and numbering the packets in accordance with the plant from which it was taken. At the end of May, when the plants were pulled up to make room for a second crop, there were eight consecutive healthy plants in each side of the north end of the house. Seed was kept from two plants only, situated in the middle of each batch. This was used in the experimental houses during the present season and, so far (September), no sign of mosaic disease has appeared. Some of the seed has also been tried in three commercial nurseries with similar results.

Tomato selection.

The batch of E.S. 1 seed was sown in new boxes in sterilised soil on December 21st, 1927, the seedlings being transferred to size "60" pots on January 23rd, 1928, and planted in two separate houses (500 per house) on March 4th.

Out of 1500 seedlings, 27 showed mosaic symptoms in the pot stage. After planting, mosaic disease did not appear until the second week in April when five plants were destroyed. Up to the third week in June, when seed was taken, 123 diseased plants had been removed. As in the case of the cucumbers, the seed from each plant was kept separate and

labelled with the plant number. That for future use was taken from twelve plants situated in the back rows and separated by at least three rows of healthy plants from spaces where infected plants had previously existed. From this seed, 3080 plants have been grown during the present season and have shown no signs of mosaic disease.

The seed, prior to selection, had been grown in the same houses since 1926, and the crop had always been infected.

Seed transmission has also occurred in the tobacco, and by rigorous destruction of diseased plants a clean stock of seed has been obtained for experimental purposes. Similar work is being carried out with petunia seed.

The possibility of reinfection from the soil cannot be neglected but it can readily be prevented by soil sterilisation either with formaldehyde or by steam.

The occurrence of aphis on cucumbers and tomatoes under glass is rare and, while white-fly (*Trialeurodes vaporariorum* Westw.) has been shown to transmit mosaic disease on one occasion (2) it does not appear to be a dangerous transmitter. The red spider mite is suspected of carrying infection from one season to another, but no definite proof is yet available.

Clean stocks of cucumber plants are not likely to become reinfected from plants other than the cucumber, for no alternative host has been found in this country.

In view of the above results, it is considered that under the glasshouse conditions of this country tomato and cucumber mosaic diseases should be kept under control by this method of cleaning infected stocks.

Summary.

1. Evidence is submitted to show that mosaic disease of both the cucumber and the tomato is transmitted by the seed.

2. Stocks of infected seeds have been cleaned by early and careful roguing of diseased plants.

3. There is every reason to believe that in this country the incidence of this disease in glasshouses can be reduced to small proportions, if not entirely prevented, by the use of clean seed.

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THE "MATURATION PERIOD" OF THE TOMATO PLANT

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(With 4 Text-figures.)

The fact that certain fruits of the tomato, mainly the apical fruits on long trusses, develop and ripen very slowly led to an investigation of the development periods of all the tomatoes produced by a single plant.

Notes were made of the date the flower opened and the date the fruit from the same flower was picked for market; the number of days

Fig. 1.

A. 1st stage: flower beginning to open.
B. 2nd stage: flower partially open.
C. 3rd stage: flower fully opened.

between being termed the "Maturation Period." Obviously this period cannot be determined exactly because of the possible error at each end. In general, tomato flowers showing the first signs of opening on any particular day are fully open at some time during the following morning, and the error at this end of the period is only a matter of a few hours.

Table I.

"*Maturation period*" *of plants, var. Ailsa Craig* (Balch).

	Truss 1				Truss 2				Truss 3				Truss 4				Truss 5				Truss 6				Truss 7			
Plant No.	97	98	99	100	97	98	99	100	97	98	99	100	97	98	99	100	97	98	99	100	97	98	99	100	97	98	99	100
Fruit No. 1	58	63	65	62	61	61	66	66	63	58 60	67	63	64	58 65	62	57	63	—	60 60	63	—	71	80	—	63 66	—		—
2	55	61	62	62	62	62	67	67* 64	62	60 66	65	63	64	58 65	63	62	63	58	63 63	63	—	—	72	—	60 83	—		—
3	59	62	63	66	60	63	69	66 66	64	60 68	66	63	67	67 63	64	63	61	60	65 58	63	—	72	71	—	64 82	—		69
4	57	63	67	66	61	64	67	65 67	66	64 70	70	65	66	68 —	67	66	61	69	82 —	67	75	73	84	—	63	79		72
5	58	66	68	68	60	61	67	67 68	67	66 83	73	67	68	74 —	66	65	64	71	97 —	—	—	72	—	—	—	77		92
6	57	67	67	69	59	62	73	103 102	67	67 —	—	69	67	82 80	79	65	72	75	102 —	—		—	—	—	—	75		—
7	59	64	67	69	62	60	74	— —	69	67 —	—	70	70	80 78	109	67	78	73	—	—					—	78		—
8	58	66	71	70	61	72	105	— —	68	107 —	—	69	93	— —	—	—	78	71	—	—						—		—
9	55	67	73	72	62	71	—	—	71	— —	—	88	95	— —	—	82	94	88	—	—								
10					99	70			108	— —	—	—	97		—	97	99	—		—								
11					—				—	—		—	100		—	97	—	—										
12																												
Average	57	64	67	67	65	65	73	73	70	69	68	68	77	70	73	74	73	71	72	64	75	72	77	—	69	77		77

* Two figures occurring for the same fruit number indicate that the truss is branched.

"*Maturation period*" *of plants, var. Ailsa Craig* (Balch).

Plant No.	Truss 1				Truss 2				Truss 3				Truss 4				Truss 5				Truss 6				Truss			
	101	102	103	104	101	102	103	104	101	102	103	104	101	102	103	104	101	102	103	104	101	102	103	104	101	102	103	104
Fruit No. 1	56	61	62	63 63	63	62	65	67	60	62	63	62	56	60	61 61	65	63	60 60	63	63	—	—	—	—	64	67	72	64
2	54	62	62	65 63	62	66	64	65	64	65	60	60	58	62	62 62	66	63	65 63	63	63	—	—			—	83	—	62
3	55	62	62	68 63	62	64	64	65	63	67	63	62	58	63	63 66	65	65	65 62	63	68	64	—			—	—	—	70
4	52	63	62	79 65	57	62	64	63	65	67	67	62	60	63	69 63	68	63	— 70	63	63	60	—			64	—	68	—
5	55	67	63	115 67	60	66	66	65	65	70	68	65	61	66	74 70	66	95	73 87	69	—	60	—			80	—	66	—
6	56	66	64	— 69	62	66	65	67	64	71	67	65	64	69	85 73	70	93	86 —	80	62						—	82	—
7	58	68	67	— —	63	65	76	66	65	88	69	72	66	77	98 —	73	100	78 —	88	84							79	—
8	58	67	74		70	67	77	105	65 67	94	68	96	93	77	— —	99	100	— —	101	82							—	
9	64	67				67	109	—	64 65	—	99	—	95	91	— —	104	98	— —	—	85								
10								—		—	—	—	95	—	— —	106	—		—	—								
11											—		—	—		—			—									
12											—																	
Average	56	65	64	71	62	65	72	70	64	73	70	68	73	70	70	75	82	70	74	71	61	—	—	—	70	75	73	65

Table II.

"Maturation period" of plants, var. E. S. 1.

	Truss 1						Truss 2						Truss 3						Truss 4						Truss 5					
Plant No.	63	64	65	66	67	68	63	64	65	66	67	68	63	64	65	66	67	68	63	64	65	66	67	68	63	64	65	66	67	68
Fruit No. 1	—	51	48	50	50	54	55	52	51	48	51	54	54	51	54	57	58	53	49	51	108	53	56	55	55	—	53 89	54	55	85
2	53	49	46	49	52	—	53	56	49	51	50	56	52	55 59	53	57	56	53	50	51	53	56	59	88	52	53	55	54	52	76
3	53	55	51	48	51	56	55	54	50	55	51	57	54	91 55	52	56	55	57	54	56	54	53	57	80	56	54	82 —	55	89	79
4	56	54	55	52	51	59	56	54	57	55	52	56	55	92 94	54	60	54	56	55	58	54	—	79	83	57	54	— —	63	57	82
5	56	56	54	52	53	57	57	54	57	55	55	60	55	98 — —	—	59	73	58	83	63	69	86	85	80	—	55	— —	—	85	86
6	58	57	60	57	57	58	57	57	58	57	55	59	56	91 132 —	—	91	—	88	83	90	72	133	93	84	—	—	— — —	86	80	130
7	63	59	59	56	58	61	—	62	60	86	—	59	59	92 — 137	—	89	—	87	85	89	—	104	93	80	—	—	— —	93	—	
8	61	60	58	—	58	64	94	53	57	56	—	98	85	— — —	—	96	99	154	84	106	92	95	105	79	86	74	— —	88	—	
9	61	112	—	82	68	62	97	103	106	102	—	96	88	— — —	—	91	—	142	98	—	89	106	104	146	—	—	— —	—	—	
10		105	—			74			105	—	—	145	103	— — —	—	89	98	141	—	—	—	113	—	131	—	—	— —	—	—	
11		—	—			—			—			—	—		—		—	139	—	—	—	—	—	—		—				
12		—	—						74			—			—			—	—		—	—								
13									—																					

Plant No.	Truss 6: 63	64	65	66	67	68
Fruit No. 1	—	Truss missed	Truss missed	—	—	58 —
2	—			—	52	— 60
3	—			—	80	57 —
4	65			—	57	63 64
5	66			—	139	68 63
6	—			—	70	74 —
7				—	78	— —
8					84	
9					—	
10					—	

Plant No.	Truss 7: 63	64	65	66	67	68
Fruit No. 1	61	—	—	64	—	79
2	63	—	64	63	—	79
3	—	66	—	63	—	—
4	66	62	—	71	61	—
5	67	68	67	70	61	—
6	64	66	—	70	—	—
7	63	67	68	—		—
8			—	—		
9						
10						

Plant No.	Truss 8: 63	64	65	66	67	68
Fruit No. 1	63	—	62	—	—	—
2	61	64	64	—	—	64
3	66	—	63	—	61	90
4	—	69	—	—	—	64
5	68	—	66	68	—	—
6	62	68	100	—		75
7	—	67	71	—		—
8	73	67	75	67		
9	—		87	—		
10	—			—		

Plant No.	Truss 9: 63	64	65	66	67	68
Fruit No. 1	63	—	63	Truss missed	65	63
2	—	68	61		67	62
3	—	67	66		64	61
4	—	68	—		63	—
5	66	66	73		—	67
6	—	76	—			71
7	—	73	78			
8	—	78	72			
9	—	—				
10						

Plant No.	Truss 10: 63	64	65	66	67	68
Fruit No. 1	63	Truss missed	—	Truss missed	62	Truss missed
2	—		65		59	
3	—		68		—	
4	—		—		68	
5	—		—		—	
6	—		74		—	
7	—		—		72	
8	—		73		—	
9	—		73			
10			73			

Fig. 1 illustrates the condition of the flower regarded as being open for the purpose of these experiments.

The date of picking allows of much greater error, although, whenever possible, fruits were picked when the first tinge of pink appeared. It

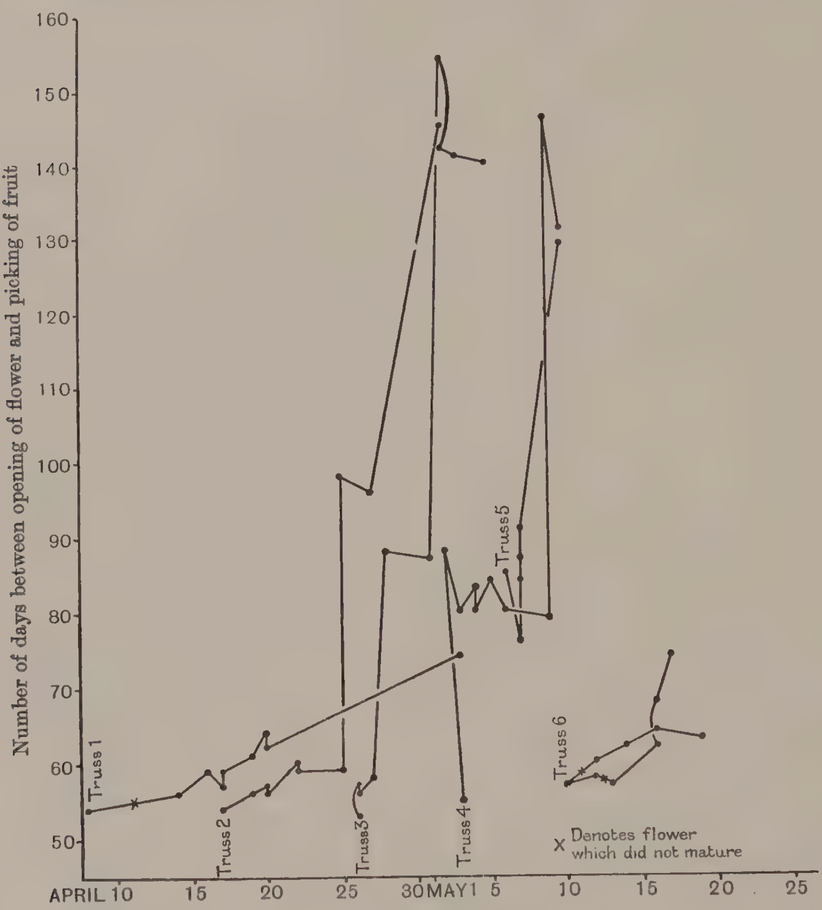


Fig. 2.

may happen, however, that fruits too green for picking on Saturday should have been taken on Sunday, but instead were left until Monday. It is estimated, therefore, that the error in picking may be as much as 2 days. This error is considered almost negligible in relation to the long maturation period of about 60 days.

Table I provides data from eight plants of the variety E.S. 1[1] growing in the same house under the same manurial and cultivation treatment. Table II relates to six plants of Balch's Ailsa Craig in another house.

It will be seen that every fruit on a plant does not develop in the same number of days, but that, in general, the maturation period lengthens as the plant ages and as the truss lengthens. When a truss bears a large number of fruits a considerable lengthening of the maturation period occurs after the sixth, seventh or eighth fruits. This is shown particularly well in the case of Plant 68, Truss 3, where the fifth fruit took 58 days to develop, the sixth 88 and the eighth 154 days. Sometimes this occurs closer to the base of the truss, as in Plant 68, Truss 4, where the first fruit developed in 55 days and the remaining fruits in 79 days and over.

This sudden lengthening of the maturation period is probably shown better in Fig. 2.

VARIETAL DIFFERENCES.

The variation in length of maturation period shown by different varieties is illustrated in Table III, two plants being examined in each variety.

Table III.

Average length in days of "maturation period."

Variety		Truss 1		Truss 2		Truss 3		Truss 4		Truss 5	
		No. of fruit	Av. No. of days per fruit	No. of fruit	Av. No. of days per fruit	No. of fruit	Av. No. of days per fruit	No. of fruit	Av. No. of days per fruit	No. of fruit	Av. No. of days per fruit
Ailsa Craig	1	11	60·1	19	65·5	9	61·1	13	64·8	11	67·0
Ailsa Craig	2	10	59·2	11	64·3	15	70·1	14	63·0	8	66·1
Comet	1	7	58·1	6	55·8	8	102·9	8	76·0	9	83·2
Comet	2	6	62·5	10	84·4	5	80·6	3	71·3	5	71·0
Manx Marvel	1	9	67·3	17	83·0	11	74·3	11	73·9	—	—
Manx Marvel	2	10	63·5	12	65·6	12	60·5	11	65·9	—	—
Masterpiece	1	8	62·6	10	62·8	11	78·8	10	84·2	8	84·0
Masterpiece	2	7	65·4	9	66·2	8	72·7	7	71·5	8	79·6
Tuckswood	1	7	62·5	11	71·4	6	76·0	5	72·4	—	—
Tuckswood	2	7	59·8	8	65·9	8	68·5	8	78·7	14	84·4

It will be seen that Ailsa Craig, a thoroughly reliable commercial variety, shows the most uniform maturation period of those tested, while Tuckswood, another good variety, comes second. Comet, an old favourite, but one which crops irregularly and is less reliable than either of the former, shows a tendency to ripen slowly on some trusses. In

[1] A hybrid selected from the progeny of Ailsa Craig × Blaby and the first variety raised by the Experimental Station, Cheshunt.

commerce, Manx Marvel shows a good deal of variation between individuals, and cannot be regarded as a pure line strain, but would probably pay for careful selection. This variation is seen between the two plants examined. Masterpiece is a variety seldom cultivated. It is abnormally short jointed and stunted in habit, with tight trusses of irregular shaped fruits. It has several good points worth noting by the plant breeder, but needs selection.

An interesting development of the work occurred in 1926, when by good fortune one of the plants proved to be an exceptionally strong and prolific individual of Ailsa Craig, as is found occasionally in this variety. This plant yielded 12 lb. of fruit instead of the usual 6–7 lb., which is good commercial average.

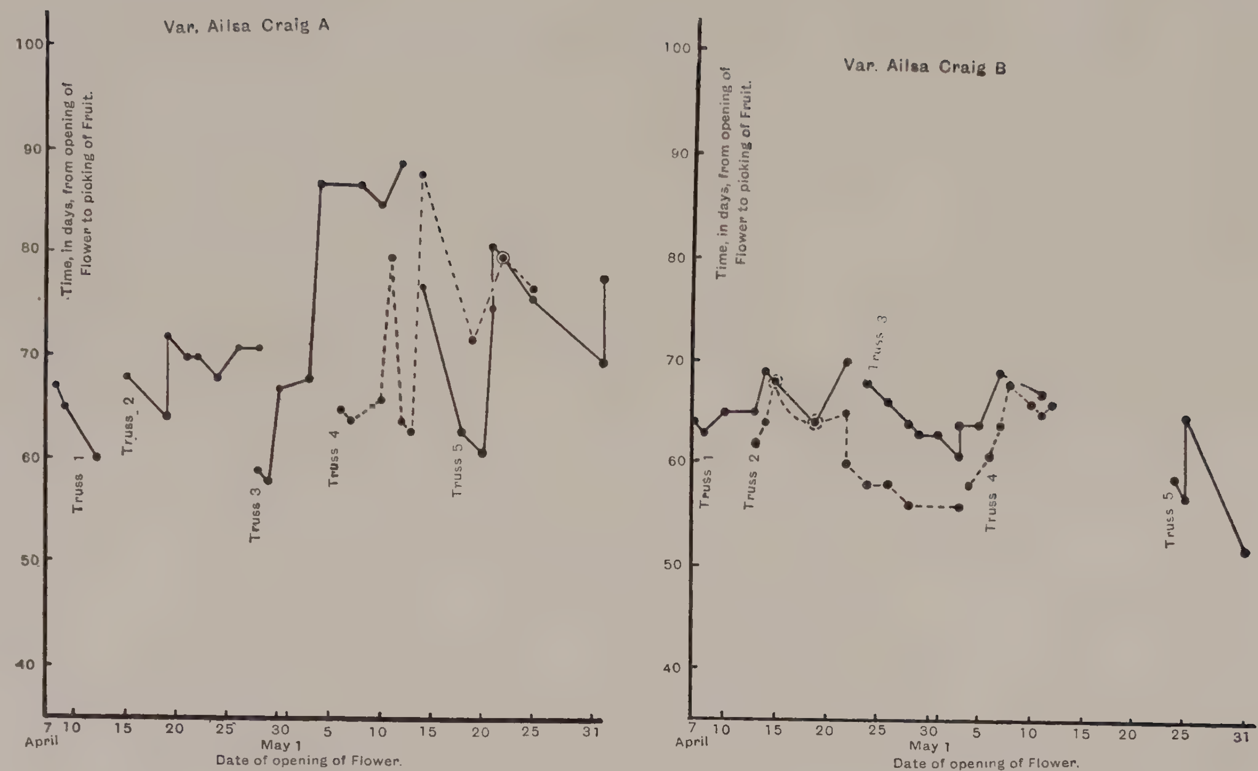
Table IV.

Truss 1			Truss 2			Truss 3			Truss 4			Truss 5		
	Length, in days, of maturation period			Length, in days, of maturation period			Length, in days, of maturation period			Length, in days, of maturation period			Length, in days, of maturation period	
Fruit No.	Plant A	Plant B	Fruit No.	Plant A	Plant B	Fruit No.	Plant A	Plant B	Fruit No.	Plant A	Plant B	Fruit No.	Plant A	Plant B
1	67	64	1	68	62	1	59	68	1	65	58	1	77	—
2	67	64	2	64	64	2	58	66	2	64	58	2	63	—
3	65	64	3	72	68	3	58	64	3	66	61	3	61	—
4	60	63	4	70	64	4	67	63	4	80	64	4	75	—
5	—	65	5	70	65	5	68	63	5	64	68	5	81	59
6	—	65	6	68	60	6	68	61	6	63	66	6	80	57
7	—	69	7	71	58	7	87	64	7	88	65	7	71	65
8	—	64	8	71	58	8	87	64	8	72	66	8	65	52
9	—	70	9	—	56	9	85	69	9	80	—	9	78	—
10	—	—	10	—	56	10	89	67	10	77	—	10	—	—
Av.	65	65	Av.	69	61	Av.	72	65	Av.	74	63	Av.	72	58

The relative figures detailed in Table IV are represented graphically in Fig. 3, Plant A being a normal type, and Plant B the exceptionally good individual. It will be seen that while Plant A shows the usual variations, the lengths of the maturation periods of all the fruits of the first five trusses on Plant B are nearly constant.

This can be seen better in Fig. 4 when the maturation period previously set out in days along the vertical arm is replaced by the actual dates of picking.

It may be assumed that an ideal plant is one in perfect balance, growing uniformly from day to day, setting all its fruits and developing them in a constant time. In this case the maturation period could be represented by the straight line A–B. Where the graph rises above A–B a retardation of the maturation period occurs and vice versa. It will be



Var. Ailsa Craig A
Time, in days, from opening of Flower to picking of Fruit.
Truss 1
Truss 2
Truss 3
Truss 4
Truss 5
100
90
80
70
60
50
40
7 April
10
15
20
25
30
May 1
5
10
15
20
25
31
Date of opening of Flower.
Var. Ailsa Craig B
Time, in days, from opening of Flower to picking of Fruit.
Truss 1
Truss 2
Truss 3
Truss 4
Truss 5
100
90
80
70
60
50
40
7 April
10
15
20
25
30
May 1
5
10
15
20
25
31
Date of opening of Flower.

Fig. 3.

Table V.

Length of maturation period, in days, of fruits of plants stopped at varying intervals.

	Truss 1						Truss 2						Truss 3						Truss 4					
Plant No.*	14	15	16	17	19	21	14	15	16	17	19	21	14	15	16	17	19	21	14	15	16	17	19	21
Fruit No. 1	61	61	63	62	—	62	59 59	62	60	62	58 60	63	—	— 66	60	61	69	61	65	—	72 —	55	—	67
2	60	59	62	64	62	61	60 54	63	60	59	62 62	62	62	61 63	63	65	65	63	—	—	70 68	—	—	68
3	62	60	59	62	64	60	60 54	60	61	65	61 63	63	60	64 63	65	63	67	66	—	—	68 —	—	65	68
4	60	59	62	60	62	64	61 —	62	63	67	64 67	61	63	63 61	64	63	70	65	—	—	— 67	58	—	—
5	63	58	62	66	66	64	65 60	63	61	65	62 66	61	64	64 —	70	68	70	63	—	—	— 67	57	63	65
6	59	58	66	63	62	—	64 —	67	64	—	— 69	61	67	74 —	73	68	—	—	61	—	67 67	63	—	73
7	60	60	63	64	63	62	—	60	62	77	63 70	61	73	— —	77	81	77	78	62	67	67 —	62	73	77
8	58	58	65	68	63	63	—	66	59	83	— 72	63	75	— —	76	78	—	—	63	64	69 —	—	—	76
9	63	65	65	66	—	65	—	63	59	81	— —	65	—	— —	76	78	—	—	56	—	67 —	—	—	73
10	70	—	—				—		—	—		68	—		—	—	—				—	—	—	—
11			—						—	—		67	—			—	—				—			—
Average	61	80	63	64	63	63	60	63	61	70	64	63	66	64	70	70	70	66	61	66	68	59	67	71

	Truss 5						Truss 6						Truss 7						Truss 8					
Plant No.*	14	15	16	17	19	21	14	15	16	17	19	21	14	15	16	17	19	21	14	15	16	17	19	21
Fruit No. 1	64 —	66	66 59	62	66	— 72	— 66	—	71 78 69	—	60 —	— —	—	58 55	— 70	— —	—	—		—		71	67 80	70 63
2	66 —	—	69 58	61	—	— —	71 69	—	— — —	—	63 63	60 54	—	59 52	— —	50 60	—	—		77		—	— 79	— 68
3	— —	—	— —	64	63	72 —	72 —	69	72 — —	68	70 63	66 58	—	60 —	64 —	55 58	57	—		76		77	71 —	77 —
4	— 91	—	— 75	66	—	— 66	74 —	70	— 76 71	—	71 —	69 62	70	63 55	67 —	55 67	59	—		75		75	84 —	75 78
5	— 91	—	— 79	68	—	— 66	— 70	76	— 83 —	—	72 65	— —	69	63 56	63 68	60 76	60	—		81		82	81 —	— —
6	— —	66	— —	65	—	66 —	— —	—	— — —	—	74 —	— —	—	69 —	66 68	65 —	63	—		77		88	93 —	— —
7	73 —	71	— —	72	—	66 —	69 —	—	— — —	—	— —	— —		69 —	69 68	— —	69	60		—		87	— —	— —
8		75	— —	—	—	— —		72	65 — 65	—	— —			— —	69 70	— —	—	65		83		84	— —	
9		—	— —	—	—	— —		—		—					— 72	— —	—			—		—		
10		—	— —	—		— —				—					— —	— —				—				
Average	77	69	67	65	65	68	70	72	72	68	67	61	69	60	68	61	62	62	—	78	—	80	80	72

* Plant 14 stopped at 1st, 4th and 6th trusses; Plant 15 at 2nd, 5th and 7th trusses; Plant 16 at 2nd, 5th and 7th trusses; Plant 17 at 3rd and 6th trusses; Plant 19 at 4th and 7th trusses; Plant 21 at 5th and 6th trusses.

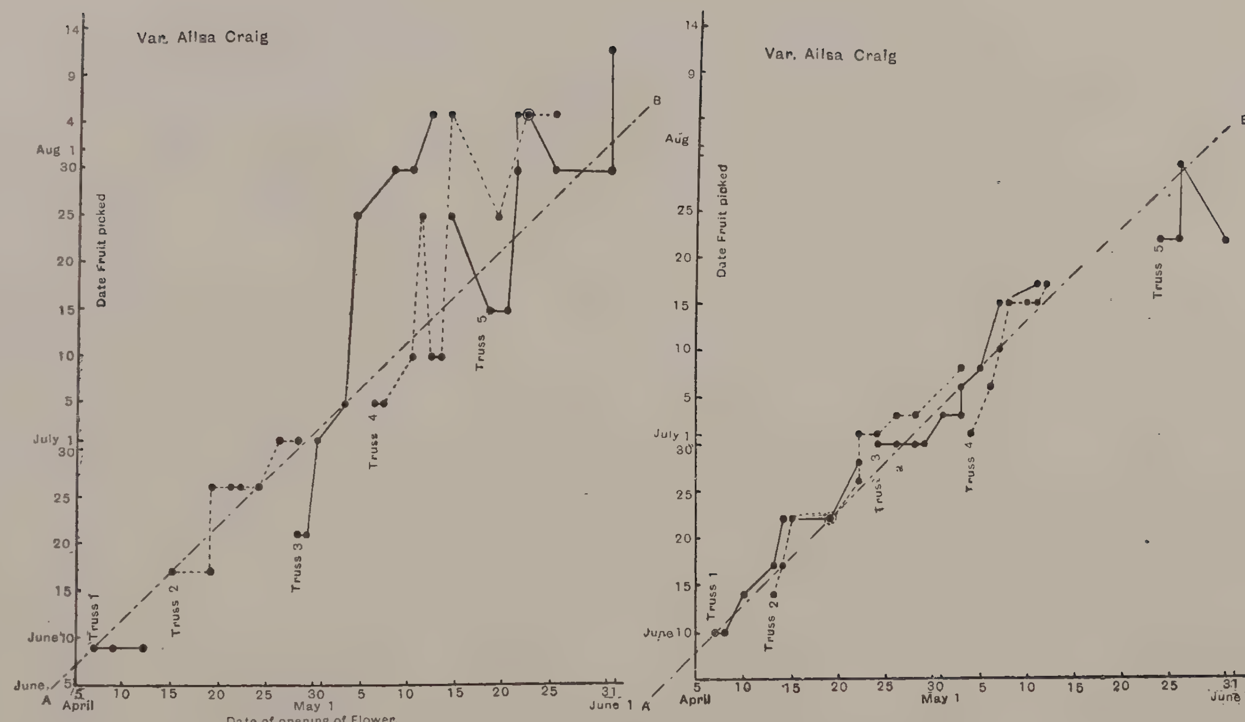

Var. Ailsa Craig
Date Fruit picked
Date of opening of Flower.
Truss 1
Truss 2
Truss 3
Truss 4
Truss 5
April
May 1
June 1
June
July 1
Aug 1
Aug
A
B

Fig. 4.

seen that Plant B approaches very closely to the ideal. The graph of the maturation period should therefore be a means of estimating the efficiency of a tomato plant, and an attempt was made to determine if it could be used in connection with the various experiments conducted at Cheshunt.

THE EFFECT OF "STOPPING" IN THE MATURATION PERIOD.

During 1927, records were made in the variety trial house on plants of variety E.S. 1, which were "stopped" at varying trusses from the first to the seventh. "Stopping" consists of cutting away the growing point so as to leave two leaves above the top truss. Growth is continued by taking up the shoot which arises in the axil of the lower leaf.

Examination of Table V, where a detailed analysis is given, shows that the maturation period approaches a constant when the plant is stopped after the first, fourth, and sixth trusses, or after the second, fifth, and seventh trusses, only 14 per cent. of the fruit taking longer than 70 days to ripen and only 30 per cent. requiring longer than 65 days. Plants stopped after either the third or fourth trusses show the usual maturation period variations.

Further, there was no appreciable gap between the opening of the last flower of the truss preceding "stopping" and the opening of the first flower of the succeeding truss. This gap is very marked in the case of plants "stopped" after the third, fourth or fifth trusses.

Other observations in connection with the manurial trials, in which only slight improvements in crop were effected by some particular treatment, did not show any marked difference in the maturation period graph of plants from the different plots, which is not surprising, because even the best plants from these trials could not be termed ideal.

In future, maturation period graphs will be prepared from plants in the experimental plots, because it seems reasonable to assume that they will provide a measure of fruit producing efficiency.

SUMMARY.

1. The period between the opening of a tomato flower and the picking of the fruit which develops from it has been termed the maturation period.

2. Usually, the maturation periods of the different fruits produced by a tomato plant vary considerably.

3. The maturation period lengthens as the plant ages, and as the truss lengthens.

4. The graph of the maturation periods of the fruits produced by a tomato plant approaches a straight line as the efficiency of the plant increases.

(*Received July 31st*, 1929.)

STUDIES IN BACTERIOSIS. XVII

ACIDIC RELATIONS BETWEEN THE CROWN-GALL ORGANISM AND ITS HOST

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(With 3 Text-figures.)

In a previous paper of this series an account is given of the reactions between some constituents of a plant sap and certain species of bacteria, including the organism causing Crown-gall, *B. tumefaciens* (1). These constituents were obtained by alcoholic precipitation of the sap, and their power to agglutinate or plasmolyse different species of bacteria was found to vary with the H-ion concentration of the solution containing them. At one definite H-ion concentration each species remained unaffected by the solution; for *B. tumefaciens* this point of non-agglutination is approximately pH 5·2.

It seemed of interest to determine whether the tissues of plants attacked by *B. tumefaciens* showed a H-ion concentration of the sap or cell contents as high as this. On staining sections of stems of tomato and *Chrysanthemum frutescens*, both of which are readily infected, with methyl red, only lignified and cuticularised cell walls and the young meristematic tissues were found to give a red coloration indicating a pH value of about pH 5·0. It is just in the meristematic regions that infection with *B. tumefaciens* and the initial stages of tumour formation probably take place, for Erwin Smith (7) and W. Magnus (2) both regard the cambium as the starting point of the tumours, and Erwin Smith adds that small tumours may also be produced by very shallow punctures into the bark parenchyma, *i.e.* by infecting the meristematic phellogen.

It has also been found by Miss Lacey, working in this laboratory, that tumours on seedling sweet peas can be obtained almost without fail by inoculating the young tissues of the hypocotyl or first node when the plumule is less than an inch long. Using the range of indicators employed by Small (6), the cortex of a section of such a plumule gives a pinkish colour with methyl red and yellow with benzene-azo α-naphthylamine, indicating a H-ion concentration of pH 5·2–4·8.

On cutting hand sections of the tumours and staining with methyl or di-ethyl red it was found that the phloem and parenchyma gave a yellow colour, indicating a H-ion concentration of about *p*H 5·8. The bacterial zoogloea in the intercellular spaces and on the surface of the tumour, however, stood out in striking contrast against this yellow background, being stained the bright red indicative of the higher H-ion concentration of about *p*H 5·0. The sections were immersed in alcohol for only a minute or two to remove air bubbles, then put in the indicator for 15 minutes or less and afterwards washed in neutral water. The coloration demonstrates the position of the threads with marked precision, the only other elements showing a pink colour being the lignified vessels, and any sclerenchyma present; the youngest meristematic cells, though pink, are less brightly stained. Unfortunately the colour fades in a day or two, and diffuses out if the sections be kept in glycerine.

Figs. 1 and 2, taken from hand sections of young tumours on pea seedlings, show the brightly coloured zoogloeal threads ramifying among the enlarged and dividing cells, and resemble closely the drawings of tumour tissue figured by Riker (Plate III, fig. B, and Plate IV, fig. D) (4), and by Robinson and Walkden (Plate V, fig. 9, and Plate VI, figs. 19 and 21) (5). The intercellular bacterial threads of a tumour strand in a tomato stem were also clearly marked, as shown in Fig. 3.

The comparatively high H-ion concentration of these strands seems rather remarkable; for as Walkden (8) observes, Erwin Smith's classification of this organism as an acid producer is "hardly justifiable," slight acidity being indicated only at the end of the third week in litmus peptone with 1 per cent. dextrose and sucrose. Erwin Smith's view is evidently based on the fact that he found acetic and formic acid as well as ammonia and aldehyde produced by the organism in flasks of tap water containing peptone and grape sugar, and he attributes the abnormal development of the host cells affected by the parasite to the presence of these substances (7).

It seems possible, however, that the bacteria may stimulate the host cell and cause it to secrete acid which is taken up by the mucilaginous strands. The growth and multiplication of the organism would not be checked thereby, as it is resistant to acids and grows readily at a *p*H of about 5·0.

A red coloration of the walls of the tumour cells can be observed close to the bacterial threads, being most marked in the middle lamella.

Robinson and Walkden (5) point out that the walls of the host cells with which the bacteria come in contact stain deeply with Sudan III

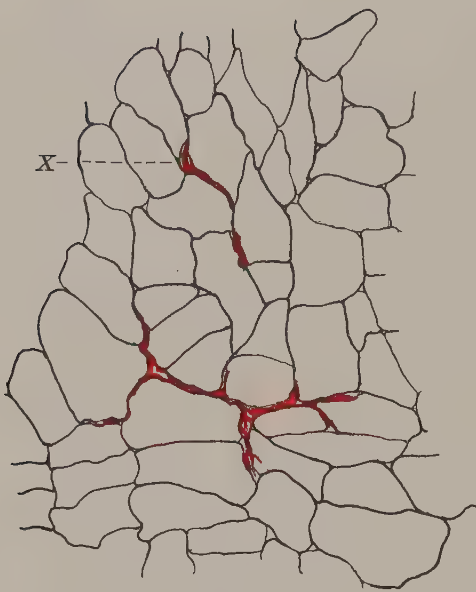

Fig. 1.

Fig. 2.

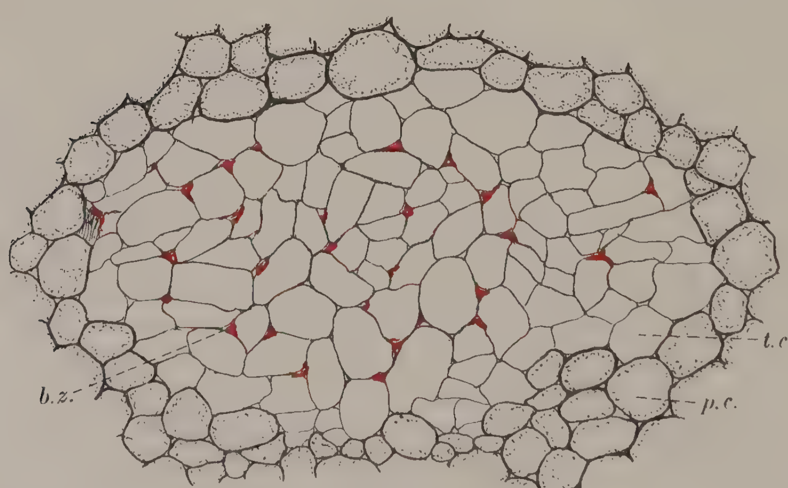

Fig. 3.

Fig. 1. Bacterial zoogloea ramifying among dividing cells in a tumour on a seedling sweet pea. *X*. Bacterial strand which could be traced to the exterior of the gall.

Fig. 2. Bacterial threads in an intercellular space among enlarged parenchymatous cells.

Fig. 3. Tumour strand in the pith of a tomato stem. *b.z.* bacterial zoogloea, *t.c.* tumour cell, *p.c.* pith cell.

and Scharlach R, and merely turn brown with strong sulphuric acid, instead of being swollen and dissolved like ordinary cellulose walls. These are reactions which usually accompany the suberisation of cell walls exposed to the air by wounding, and Priestly and Woffenden regard such suberised walls as containing free acid, since they stain red with methyl red (3).

This suberisation of the cell walls in contact with the bacterial zoogloea is accompanied by growth and division of the host cells (Fig. 1) which appear to return to a semi-meristematic condition at those points where the bacterial stimulation is strongest. It seems just possible that the process of tumour formation may be regarded as due to an irregular and progressive healing reaction essentially the same as that which takes place after the wounding of plant tissues.

In conclusion the writer wishes to express her thanks to Prof. Blackman and Dr Paine for their kind interest and criticism in the preparation of this paper.

SUMMARY.

1. The H-ion concentration of plant sap most favourable to the growth of *B. tumefaciens* has been shown in a previous paper to be about *p*H 5·2. This H-ion concentration characterises meristematic tissue where tumours due to *B. tumefaciens* originate.

2. The bacterial zoogloea present within and on the surface of the tumours gives the red colour with methyl red which indicates approximately the same value, *p*H 5·2.

3. The cell wall in contact with the bacterial zoogloea appears to be suberised; and it is suggested that the process of tumour formation is due to healing reactions similar to those which take place in wounded plant tissue.

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THE SOIL FUNGI OF THE DOVEY SALT MARSHES

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(With 11 Text-figures.)

CONTENTS.

INTRODUCTION.

THIS research was undertaken at the suggestion of the late Prof. Yapp with the object of finding out whether any fungi were present in the soil of the Dovey Salt Marshes at Ynyslas, near Borth.

Prof. Yapp had studied intensively the vegetation of the Dovey estuary, and had published in 1917 and 1921 some of his researches on the subject.

The soil of this salt marsh is developed on a layer of estuarine silt overlying peat, and would hardly be considered a favourable medium for the growth of fungi: for the most part in the area investigated it was found to be a badly aerated, stiff tenacious clay, alkaline in reaction (pH 8) with a high water content, due mainly to periodical inundation by tidal salt water.

The vegetation covering the marsh shows a marked zonation, five plant associations[1] being readily distinguished. These are:

1. Salicornietum Europaeae. Lowest.
2. Glycerietum maritimae. ·00 ft. to 1·3 ft.
3. Armerietum maritimae. 1·00 ft. to 2·6 ft.
4. Festucetum rubrae. Highest.
5. Juncetum maritimi. ,,

[1] The ecological terminology used here is taken from Yapp (1).

Very few soil samples from the Salicornietum were examined, since owing to the prolonged submergence of this area—twice daily—only a few poorly grown plants of *Salicornia Europaea*, with here and there a little algal vegetation, managed to withstand its hardships.

It was not surprising therefore to find that on a substratum so lacking in nutriment of the nature of humus, that fungi, although present, were few and far between.

However, in the Glycerietum and Armerietum a number of interesting species was found, and bacteria, many species of which were of brilliant hue, were abundant.

Soil samples were taken chiefly from the Glycerietum and Armerietum. These two associations form a fairly compact sward which has been used for years by the farmers in the district as perennial pasture land for sheep. It is said that sheep prefer the salt marsh pastures to ordinary ones, and thrive on them. At the outset of this investigation a few samples were taken from the Juncetum and Festucetum, but it was thought best to concentrate on the Glycerietum and Armerietum.

The Glycerietum consists of nearly pure *Glyceria maritima*, but occasional stunted plants of *Salicornia Europaea* occur, and in the highest parts of the zone, plants of *Armeria maritima* are met with.

The Armerietum is nearly pure *A. maritima*, but in the lowest parts of the zone, *G. maritima* may occasionally be subdominant.

METHOD OF TAKING AND OF DEALING WITH SOIL SAMPLES.

Since the vegetation of the salt marsh at Ynyslas seemed fairly uniform in character, the soil samples were all taken within an area of about 15 or 20 square yards of one another, in the two different zones. The idea was that by examining a large number of samples taken from such an area, a fungus flora representative of the salt marsh might be obtained, if such existed.

Samples were taken during various months of the year, because although desirable, it was impossible to deal with samples taken at regular intervals. The first samples were taken in July 1926 and subsequent ones in February, June, September, and November 1927, and in May and June 1928.

The samples were obtained in the following way. A wedge-shaped sod, one side being vertical and about 8 inches in depth, was taken out of the sward and later replaced. In the depression thus made, a thin slice was cut off the vertical side with a sterile knife and short sterile tubes were pushed in it at depths of $1\frac{1}{2}$ and $3\frac{1}{2}$ inches, and samples of

soil taken out; a sterile cork was inserted in each tube immediately it was withdrawn with its sample from the soil. Time did not permit of samples taken from a lower depth than $3\frac{1}{2}$ inches being examined, and since various investigators (Goddard (2), Waksman (3), Brierley (4), etc.) have found that fungi are most numerous in the first 6 inches of soil, this investigation was limited to samples taken well within that range.

Direct microscopic examination of the soil samples did not yield much information. Conidia and fragments of hyphae were rarely met with, and then usually only in association with organic matter such as dead or dying roots, stems, and leaves: however, by using pure culture methods a number of fungi were isolated.

For inoculation purposes approximately 1 gm. of soil was dropped into an Erlenmeyer flask containing 50 c.c. of sterile tap water and shaken for half an hour. By means of a dropping tube 0·5 c.c. of this suspension was then introduced into the tube and Petri dish cultures at hand—for most cultures were inoculated on the salt marsh, and as it happened, when a good stiff sea breeze was blowing. Some tubes and Petri dishes were also infected by planting very tiny portions of soil taken on the end of a sterile scalpel directly on to the medium. For controls, tubes and Petri dishes were left open on the marsh while the cultures were being inoculated.

The cultures inoculated with soil always grew more rapidly than those cultures which received the soil suspension inoculation. According to Waksman (3, 5), this is due to the continued growth of those fungi which are in the active mycelial condition at the time of inoculation. This was confirmed by Brown (6) and by McLennan (7).

All apparatus and media used were prepared and packed in the botanical laboratory under sterile conditions, and then transferred to the marsh, where the soil samples were taken, and tubes and Petri dishes infected.

Media used.

In certain trial experiments various media were used, including some to which an extract of salt marsh soil was added, but better results were obtained by using the following media.

Bread agar.

Tap water 500 c.c.

Bread. Sufficient to make 500 c.c. of water a creamy consistency.

Agar. 7·5 gm.—enough to make medium set.

Adjusted to pH required before being sterilised in autoclave.

Potato agar.

Tap water 500 c.c.

Potatoes. Sufficient cooked, mashed potatoes to make water a creamy consistency.

Agar. 7·5 gm.—enough to make medium set.

Not adjusted to any *p*H.

Raisin agar.

Tap water 1000 c.c.

Raisins 60 gm.

Agar 25 gm.

Adjusted to required *p*H.

In about half the cultures used the medium was adjusted to *p*H 5 before being autoclaved. A fairly acid medium was used, because it is generally understood that this has the advantage of keeping down colonies of bacteria. However, acidity seemed to have little effect in diminishing the growth of salt marsh bacteria in culture. The remaining tubes and Petri dishes were adjusted to *p*H 6, *p*H 7 and *p*H 7·5.

Within 24 to 48 hours after inoculation the cultures were brought back to Birmingham. At the outset some of these were incubated at 23°and 25° C. but ordinary room temperature was found to be more advantageous, since higher temperatures favoured the growth of bacteria, some mucors and certain fungi. At ordinary room temperatures growth was sufficiently slow for most of the fungi which appeared, to be identified and subcultured. In about six cultures, and perhaps more, mycelium appeared which remained sterile even when subcultured under various conditions.

Cultures were examined at intervals of a few days; usually 10 or 14 days or even more elapsed before the colonies produced conidia or spores and it became possible to identify the species.

FUNGI FOUND IN THE SOIL OF THE SALT MARSH.

As has been frequently pointed out, culture media are selective, so those used probably were not suitable for the development of all species that were present in the salt marsh area investigated, also parasitic forms are likely to have been missed.

It is said that rigid identification is unsatisfactory because of the variation of morphological and other characters with different media: that may be true of genera like *Cladosporium* and *Fusarium*, yet many fungi isolated from the soil such as *Trichoderma Köningi* and *T. lignorum*

have sufficiently well-defined specific characters to be identified, and when monographs such as those by Thom and Church (8) on *Aspergillus*, and by Thom (9) on *Penicillium* are available for other very variable genera, identification of these will be less vague.

During this investigation 48 fungi were isolated: but from the fact that, after certain calculations were made, it was found that a suspension of 1 gm. of waterlogged soil in 50 cc., only an average of 30 colonies to the gram appeared, it would seem that fungi are not abundant in the salt marsh. For comparison, using cultivated soil (waterlogged) taken from the university grounds, Birmingham, after heavy rains, similar methods gave an average of 260 colonies to a gram of soil.

However, it has been pointed out by Brierley[1], Conn (10) and others that counts of colonies appearing in Petri dishes may not necessarily indicate relative abundance of fungi active in the soil, it may merely indicate the activity of one or more fungi in the production of reproductive bodies.

Judging from the number of fungi obtained in the samples collected in six different months of the year, June is the most favourable month. A temperature reading at a depth of $3\frac{1}{2}$ inches in the salt marsh in June 1928 (12 a.m.) gave 19·5° C.—a very favourable temperature for the growth of many soil fungi.

Generally speaking, the same species were common to both the Glycerietum and Armerietum: also the species found at a depth of $3\frac{1}{2}$ inches were for the most part the same as those at a depth of $1\frac{1}{2}$ inches, whether in Glycerietum or Armerietum.

The following species of fungi were isolated from soil samples collected on the dates given:

July 19th, 1926.

- Fusarium oxysporium var. resupinatum (Sherb.)
- Botrytis pyramidalis (Sacc.)
- Hormodendron cladosporoides (Fres.) (Sacc.)
- Stysanus medius (Sacc.)
- Mucor circinelloides (Van Tieghem)
- Diplococcum resinae (Corda)
- Echinobotryum laeve (Sacc.)
- Torula allii (Harz)
- Mortierella pusilla (Oud.) var.
- Stachylidium extorre var. majus (Berl.)
- Fusidium viride (Grove)
- Penicillium hyphomycetis (Sacc.)
- Acrostalagmus cinnabarinus (Corda)
- Cephalosporium acremonium (Corda)
- Periconia felina (E. March.)
- Botrytis cinerea (Pers.)
- Perithecia of Aspergillus fumigatoides (B. and S.)

[1] See Brierley (4), p. 123.

February 15*th*, 1927.

Botrytis pyramidalis (Sacc.)
Aspergillus versicolor (Vuillemin)
Mucor sphaerosporus (Hagem)
Penicillium hyphomycetis (Sacc.)
Fusarium oxysporium var. resupinatum (Sherb.)
Spicaria griseola (Sacc.)
Acrostalagmus albus (Preuss)
Cephalosporium acremonium (Corda)
Periconia felina (E. March.)
Cephalosporium humicola (Oud.)
Monilia pruinosa (Ck. and Mass.)
Acrostalagmus cinnabarinus (Corda)

June 1927.

Acrostalagmus cinnabarinus (Corda)
Mucor circinelloides (Van Tieghem)
Cephalosporium humicola (Oud.)
Fusarium oxysporium var. resupinatum (Sherb.)
Hormodendron cladosporoides (Fres.) (Sacc.)
Perithecia of Aspergillus fumigatoides (B. and S.)
Mucor sphaerosporus (Hagem)
Cladosporium lignicolum (Corda)
Trichoderma lignorum (Harz)
Penicillium hyphomycetis (Sacc.)
Acrostalagmus albus (Preuss)
Rhizopus nigricans var. minor (Jensen)
Penicillium expansum (Link.)
Aspergillus versicolor (Vuillemin)
Periconia felina (E. March.)
Fusidium viride (Grove)
Trichosporium murinum (Sacc.) var.
Stysanus medius (Sacc.)
Trichoderma Köningi (Oud.)
Fusarium anguioides (Sherb.)

September 19*th*, 1927.

Fusarium oxysporium var. resupinatum (Sherb.)
Torula allii (Harz)
Penicillium claviforme (Bainier)
Stysanus medius (Sacc.)
Perithecia of Aspergillus fumigatoides (B. and S.)
Echinobotryum atrum (Corda)
Cephalosporium humicola (Oud.)
Trichoderma lignorum (Harz)
Mucor circinelloides (Van Tieghem)
Periconia felina (E. March.)
Acrostalagmus cinnabarinus (Corda)
Acrostalagmus albus (Preuss)
Penicillium hyphomycetis (Sacc.)

November 15*th*, 1927.

Torula allii (Harz)
Penicillium claviforme (Bainier)
Perithecia of Aspergillus fumigatoides (B. and S.)
Chaetomium crispatum (Fckl.)
Cephalosporium humicola (Oud.)
Trichoderma lignorum (Harz)
Fusarium oxysporium var. resupinatum (Sherb.)
Aspergillus versicolor (Vuillemin)
Citromyces glaber (Wehmer)
Stysanus medius (Sacc.)
Mortierella pusilla (Oud.)

May 17*th*, 1928.

Mucor racemosus (Fres.)
Fusarium oxysporium var. resupinatum (Sherb.)
Penicillium decumbens (Thom.)
Sporotrichum laxum (Nees.)
Fusarium anguioides (Sherb.)
Mucor microsporus (Namyslowski)
Oospora lupuli (Mass. in litt.)

Penicillium commune (Thom.)
Torula allii (Harz)
Penicillium hyphomycetis (Sacc.)
Trichoderma Köningi (Oud.)
Trichoderma lignorum (Harz)
Hormodendron cladosporoides (Fres.) (Sacc.)

June 21st, 1928.

Penicillium expansum (Link.)
Mucor microsporus (Namyslowski)
Fusarium oxysporium var. resupinatum (Sherb.)
Trichoderma Köningi (Oud.)
Trichoderma lignorum (Harz)
Periconia felina (E. March.)
Torula allii (Harz)
Aspergillus versicolor (Vuillemin)
Oospora lupuli (Mass. in litt.)
Clasterosporium carpophilum (Lév.)
Citromyces glaber (Wehmer)
Zygorhynchus Moelleri (Vuillemin)
Penicillium hyphomycetis (Sacc.)
Sporormia intermedia (Awd.)
Hormodendron cladosporoides (Fres.) (Sacc.)
Mucor sphaerosporus (Hagem)
Aspergillus versicolor (Vuillemin)
Aspergillus flavus (Link.)
Perithecia of Aspergillus fumigatoides (B. and S.)
Periconia felina (E. March.)
Aspergillus fumigatus (Fres.)
Fusidium viride (Grove)
Torula lucifuga (Oud.)

As has already been mentioned, only a few soil samples were collected in the Salicornietum. From these, 12 fungi were isolated, all of which, with the exception of *Chaetomium spirale* and *Macrosporium commune*, appear in the list given for Glycerietum and Armerietum.

List of fungi isolated for Salicornietum.

Chaetomium spirale (Zopf)
Cephalosporium acremonium (Corda)
Mortierella pusilla (Oud.)
Macrosporium commune (Rabh.)
Hormodendron cladosporoides (a very variable form) (Fres.) (Sacc.)
Oospora lupuli (Mass. in litt.)
Stachylidium extorre var. majus (Berl.)
Torula allii (Harz)
Monilia pruinosa (C. and M.)
Cladosporium lignicolum (Corda)
Torula lucifuga (Oud.)

Comments on the fungi isolated.

Among the 48 species recorded are seen species (*Trichoderma Köningi*, *T. lignorum*, *Acrostalagmus cinnabarinus*, *A. albus*, *Hormodendron cladosporoides*, *Penicillium decumbens*, *P. expansum*, *Aspergillus fumigatus*, *A. flavus*, *Chaetomium crispatum*, *Zygorhynchus Moelleri*, *Mucor circinelloides*, *M. sphaerosporus*, etc.) which have been repeatedly isolated from soil in various parts of the world by various investigators—Waksman (3), Jensen (11), Dale (12), Goddard (2), Oudemans and Köning and others.

It will be noted that the most common species found in the salt marsh were *Torula allii*, *Penicillium hyphomycetis* and *Fusarium oxysporium* var. *resupinatum* (Sherb.).

Fusarium oxysporium var. *resupinatum* was isolated by Waksman (3) from garden and iron soils, and is one of the *Fusaria* of potatoes (13), but there appears no record of *Torula allii* or *Penicillium hyphomycetis* as soil fungi, yet they were present in every sample of salt marsh soil.

Almost equally common were *Trichoderma lignorum*, *T. Köningi*, *Hormodendron cladosporoides*, *Mucor circinelloides* and *Periconia felina*.

Each of the following species appeared in three at least of the seven collections of soil samples investigated: *Mortierella pusilla*, *Botrytis pyramidalis*, *Cephalosporium humicola*, *Aspergillus versicolor*, *Stachylidium extorre*. *Penicillium claviforme*, *Citromyces glaber*, *Cephalosporium acremonium*, *Oospora lupuli* are species which were each recorded in two collections: while *Cladosporium lignicolum*, *Echinobotryum atrum*, *Aspergillus varians*, *Mucor racemosus*, *Clasterosporium carpophilum*, *Aspergillus fumigatus*, *Botrytis cinerea*, *Chaetomium crispatum*, *Mucor nigricans* var. *minor*, *Sporotrichum laxum*, *Trichosporium murinum* are species which were only isolated from one collection.

In the control tubes, which were left open on the salt marsh during the inoculation of the 80 to 100 tubes and Petri dishes dealt with on each visit, the following three species were seen: *Macrosporium commune*, which appeared only on one occasion and is not included in the list of species taken from the soil; *Trichoderma lignorum*, which appeared in one control tube only, yet was frequently recorded for the salt marsh soil; and *Hormodendron cladosporoides*, which was present in control tubes on two occasions and occurred repeatedly in the soil samples.

As far as the writer is aware, the following species in the above lists have not been recorded before for the British Isles:

Periconia felina (E. March.)
Fusarium anguioides (Sherb.)
Spicaria griseola (Sacc.)
Cephalosporia humicola (Oud.)
Mortierella pusilla (Oud.) var.?
Rhizopus nigricans var. minor (Jensen)
Torula allii (Harz)
Torula lucifuga (Oud.)
Diplococcum resinae (Cord.)
Fusarium oxysporium var. resupinatum (Sherb.)
Aspergillus fumigatus (Fres.)
Chaetomium crispatum (Fckl.)

The mode of growth in culture of several species of fungi met with in the salt marsh seems worthy of comment. These, instead of growing out in the close radiating mass so familiar in cultures of *Penicillium*, *Aspergillus* and others, send out, to begin with, a few branches which grow rapidly in length, fruiting as they grow; other branches follow in the same direction, coiling round the pioneer branches, and others round these until, as it were, rope-like strands are formed (Fig. 1, *a*) from which

later similar rope-like strands branch out. In this way the fungus quickly covers a whole substratum, often overwhelming and burying other species *en route*.

A fungus with this method of growth should be highly successful in dominating a substratum, and in a salt marsh sward where vegetation is not a very close compact turf, this runner method of growth must be highly advantageous to the fungus possessing it.

Fig. 1. *Mortierella pusilla* (Oud.) var.: *a*, mode of growth, ×73; *b*, sporangium, ×600; *c*, spores, ×600.

Of the 48 fungi recorded for the salt marsh, 27 are members of the Fungi Imperfecti, 14 belong to the Ascomycetes and 7 to the Mucorales.

Several septate sterile mycelia (orange, fuscous, white, etc.) appeared in cultures, and one possessing golden brown hyphae showed clamp connections and doubtless belonged to the Basidiomycetes. This was the only instance of clamp connections found during this investigation.

Echinobotryum laeve (Sacc.) (Fig. 2).

Echinobotryum laeve (Sacc.) appeared as a parasite on *Stysanus medius* (Sacc.) each time this fungus cropped up in soil cultures. The conidia were smaller than the type, being 7–8 μ × 4 instead of 12 μ × 6–7.

Appended to the description of *E. laeve* by W. B. Grove (21) is a note stating that he thought the fungus merely a young stage of *E. atrum* (Corda). So when the latter fungus (Fig. 3) also appeared in these salt marsh soil cultures, the two species were kept under observation, and it was readily seen that the beaked coarsely warted pear-shaped conidia of *E. atrum*, which are attached by their broad end to their conidiophores, are quite different from the fusoid smooth conidia of *E. laeve*; further, the hyphae, as stated in the description of the latter fungus, remain hyaline when mature. These two species of fungi are undoubtedly quite distinct from one another.

Chaetomium crispatum (Fckl.). New record.

This fungus was taken in July 1926 from the Salicornietum and in November 1927 from the Glycerietum and seems very closely allied if not identical with *C. olivaceum* (C. and Ellis) which was isolated from soil by Jensen(11), and also by Waksman(3). It is also very closely allied to *C. simile*, a coprophilous fungus recorded and figured by Massee and Salmon(19).

Periconia felina (E. March.).

The only other record of this fungus is for Belgium (on cat dung). It appeared in four or more series of soil samples and in most tubes of each series.

The salt marsh form differed from the type in having its conidiophores attenuated at the apex instead of being thickened; otherwise there was agreement. Although,

Fig. 2.

Fig. 3.

Fig. 4.

Fig. 2. *Echinobotryum laeve* (Sacc.), hyphae with clusters of conidia, ×600.

Fig. 3. *Echinobotryum atrum* (Corda), hyphae with clusters of conidia, ×600.

Fig. 4. *Periconia felina* (E. March.): *a*, mode of growth in rope-like strands, ×66; *b*, attenuated conidiophores terminating in conidial heads, ×600; *c*, conidia, ×600.

according to Marchal's description, the conidiophores are thickened at the end, in his diagrams they are attenuated, and each terminates in a swelling which looks like the beginning of the first spore: the salt marsh form resembles the diagram (Fig. 4, *a*, *b*, *c*).

This fungus had the scrambling method of growth referred to on p. 291, and rapidly covered the substratum with a mesh-work of rope-like strands of septate hyphae, all bearing simple or branched hyphae which were hyaline for a long time: each conidiophore terminated in a head of eight or ten black conidia, all held together with mucus. The conidia are oval and measure $6 \times 2{\cdot}5\text{–}3\,\mu$.

Fusarium anguioides (Sherb.). New record.

Besides the almost universally present *F. oxysporium* var. *resupinatum* (Sherb.), in one culture, June 1927, *F. anguioides* appeared. Most of the conidia were of the

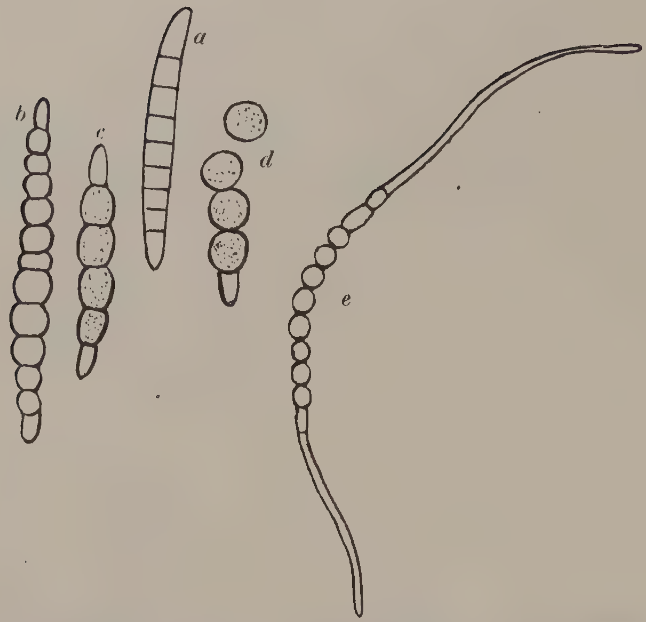

Fig. 5. *Fusarium anguioides* (Sherb.): *a*, conidium; *b* and *c*, conidia preparing to break up into unicells; *d*, conidium breaking up into unicells; *e*, germinating conidium. *a*, *b*, *c*, *d*, *e*, ×600.

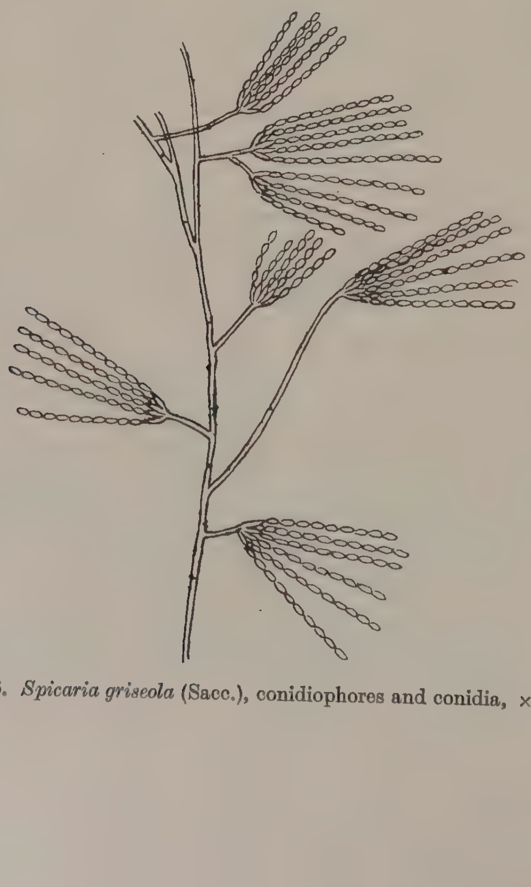

Fig. 6. *Spicaria griseola* (Sacc.), conidiophores and conidia, ×600.

extraordinary long variety (90–100 μ), five to nine septate, and were interesting from the fact that many of them broke up into unicells before germination (Fig. 5).

Stysanus medius (Sacc.). New record.

This species differs from *S. stemonites* (Corda) in its smaller conidia, the marsh form being rather small, usually 3 × 2·5 μ, occasionally 5 × 2·5 μ.

Spicaria griseola (Sacc.). New record.

In one culture a *Spicaria* closely related to *S. griseola* appeared. It differed from the type chiefly in the colour which always remained white, and in its simpler branching—the conidiophores were nearly always simple, and branched rarely and then only sparingly. The conidiophores like the type terminated in five or six short branches (sterigmata, 3 μ) to which very long chains of a septate colourless elliptical conidia (2·5 × 1·5 μ) were attached (Fig. 6).

Cephalosporium humicola (Oud.). New record.

This fungus appeared in soil samples taken in February, June and November, and had the same scrambling method of growth as *Periconia felina* and others.

Fig. 7. *Cephalosporium humicola* (Oud.): *a*, mode of growth, × 80; *b*, conidial heads on conidiophores, × 600; *c*, round conidia, × 600; *d*, oval conidia, × 600.

The spherical conidia were rather smaller than the type, being 1·5 μ in diameter instead of 2·3 to 2·5 μ, also in some cultures a variety with the conidia decidedly oval (1·5 × 1) was seen (Figs. 7, *d* and 7, *c*). Unlike the type, the conidiophores were more often branched than unbranched. The colonies were white for a long time but occasionally a rosy tint sometimes appeared in old cultures (Fig. 7).

Occasionally the conidia attained the dimensions (3–4 × 1–1·5 μ) given for *C. acremonium* (Corda) which supports the suggestion of W. B. Grove that *C. humicola* is only a form of *C. acremonium*.

Mortierella pusilla (Oud.) var.?

This fungus is closely related to *M. pusilla*, yet differs in having conidiophores shorter and narrower, sporangia not nearly so wide, and small elliptical spores (2–3·5 × 1·5–2 μ) instead of round ones. Both fertile and vegetative hyphae were narrower than the type. In its mode of growth it resembles *Cephalosporium humicola* (Fig. 1).

The type form was isolated from humus soil in Holland and is not recorded for the British Isles.

Aspergillus fumigatus (Fres.). New record.

This *Aspergillus* appeared in cultures from soil samples taken in February 1927 and June 1928, but no perithecia were obtained. It seems doubtful whether they are known.

Aspergillus fumigatoides (B. and S.).

In cultures from many soil samples black perithecia were of frequent occurrence—perithecia with rough ascospores, diameter 2·5–4, golden brown in mass—all characteristics which distinguish *A. fumigatoides* (B. and S.), a species which, according to Thom and Church (8), possesses a perithecial form closely allied to *A. fumigatus*.

The ascospores, when transferred to various media, failed to germinate.

Rhizopus nigricans var. *minor* (Jensen). New record.

This variety appeared in one soil sample taken at a depth of 3½ inches in the Armerietum. It agreed with the type. The only other record of this fungus is that of Jensen (11) for Ithaca.

Trichosporium murinum (Sacc.) var.

A species of Trichosporium very closely allied to *T. murinum* was taken from soil samples collected in June 1927. The spores are somewhat larger in size than the type, being $15\,\mu \times 5\text{–}7$ (instead of $10\text{–}12 \times 8\,\mu$), but in obovate form and in sessile

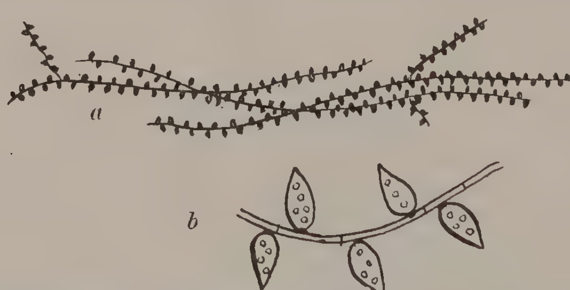

Fig. 8. *Trichosporium murinum* (Sacc.) var.: *a*, mode of growth, ×60; *b*, sessile conidia, ×600.

attachment they resemble Saccardo's figure. The hyphae were hyaline (not dark), and all were fertile, a profusion of black sessile conidia being produced in alternate succession (Fig. 8).

This fungus has a scrambling method of growth and very soon smothers a mixed culture in which it appears.

Torula allii (Harz). New record.

A fungus which has appeared in nearly every soil sample collected on the salt marsh has the characteristics of *T. allii*, which was described by Harz as parasitic on onion bulbs, in Vienna. Harz gives the colour of the conidia as brownish black,

whereas the salt marsh form has golden brown, multi-guttulate conidia, very variable in diameter (5–14 μ) (Fig. 9).

There is no previous record of this fungus for the British Isles, but probably it would be found if looked for on *Allium*.

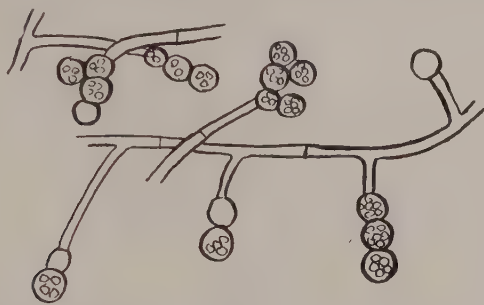

Fig. 9. *Torula allii* (Harz), conidia and conidiophores, ×600.

Mucor microsporus (Namyslowski).

This salt marsh mucor differs from the type in its shorter conidiophores (about 5 μ instead of 12–20 μ), and in its smaller sporangia about half the size.

Oospora lupuli (Mass. in litt.).

This fungus is a common pink mould on spent hops. The salt marsh form has rather smaller conidia which measure 5–7 × 2 μ instead of 7–9 × 4 μ.

Clasterosporium carpophilum (Lév.).

This fungus, known only as a parasite, forms an effused black velvety mass over the surface of the substratum on which it is cultured (Fig. 9*). The sterile and fertile hyphae are fuscous, the simple or branched conidiophores terminate in two or three

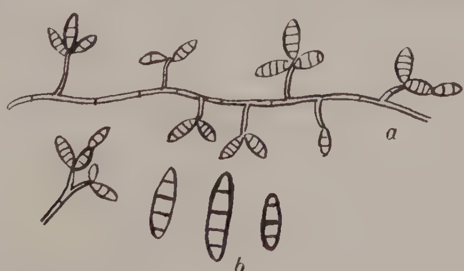

Fig. 9*. *Clasterosporium carpophyllum* (Lév.): *a*, conidia and conidiophores, ×333; *b*, septate conidia, ×666.

sessile, or nearly so, conidia, sometimes in chains of two. Conidia 20–60 μ × 8–10, 1, 2, 3, 4 and 5 septate, oval, somewhat pointed at one end.

The only British record of this fungus is by Berkeley and Broome(14) who record it as a parasite on ripe peaches (1864). Aderhold(15) has proved that it is capable of causing gummosis of prunaceous hosts.

Torula lucifuga (Oud.). New record.

A salt marsh fungus which agrees closely with the description of the *T. lucifuga*, was isolated from soil samples taken in June 1928, but only one colony was noted.

The type species was isolated from humus soils in Holland by Köning. It has not been recorded before for the British Isles.

Stysanus n.sp. or abnormal form of *Stysanus*.

This species was of an unusually large size for a *Stysanus*. It appeared in a soil sample taken at a depth of 1½ inches in the Salicornietum, June 1927.

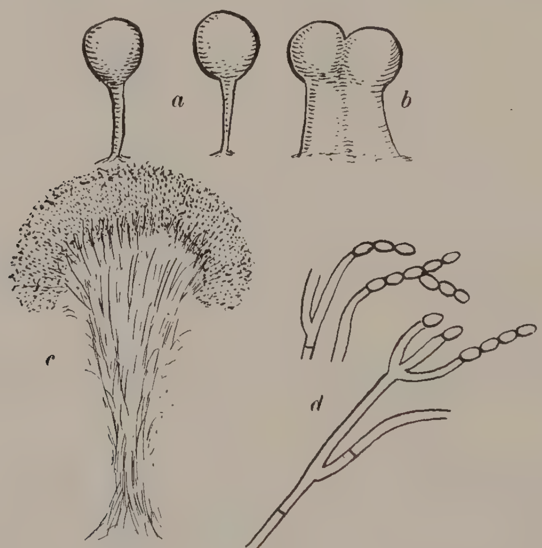

Fig. 10. *Stysanus* sp.: *a*, torch-like sporodochium, ×14; *b*, fused sporodochia; *c*, section through a sporodochium, ×53; *d*, fertile hyphae with chains of conidia from the conidial head, ×600.

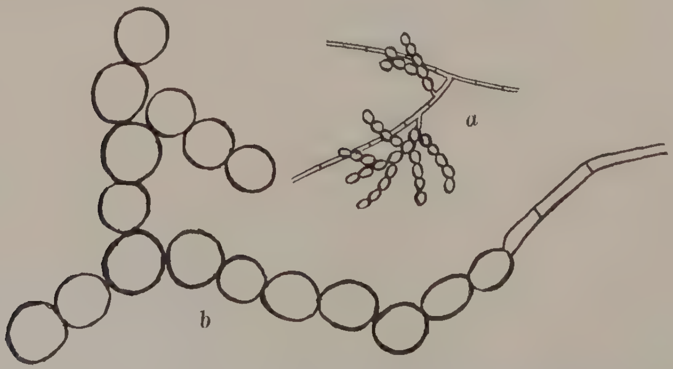

Fig. 11. *Monilia pruinosa* (C. and M.): *a*, conidia and conidiophores, ×100; *b*, conidia, ×600.

Diagnosis. Stem of stroma, shining white, simple, gradually widening upwards, height 1·2 to 2 mm., width at base 200 μ increasing to 800 μ, consisting of septate colourless hyphae; head globose, fuscous, diameter 600 μ to 2000 μ: conidia ovate, $3 \times 2{\cdot}75\,\mu$, pale fuscous, mostly in unbranched chains (Fig. 10).

The large size of this fungus, its shining white stem and torch-like form, distinguish it from any species of *Stysanus* already recorded. In the size of its conidia it resembles *Stysanus microsporus* (Sacc.) and *Stysanus Mandlii* (Mort.).

Monilia pruinosa (C. and M.).

The only other record of this fungus (Fig. 11) appears to be that by Cooke and Massee, who found it at Kew on fading leaves of Caladium. They do not give a figure. It appeared in cultures (February 1927) which were planted with minute fragments of soil from the Glycerietum, and it also was found (November 1927) in the Salicornietum (Fig. 11).

It is of interest to note that Mason(16), during her investigation of salt marsh plants, came across fragments of an endophytic fungus in the roots of *Armeria* and *Glyceria*, which very much resembled this.

FUNGI IN RELATION TO THE SOIL.

One is impressed by the fact that almost all the aforementioned fungi are found above ground as saprophytes. Thus it seems probable that many of them have been introduced into the soil by drainage or from the excrement of sheep, birds, etc., or by the wind; however, when introduced they would grow actively if they met with suitable nutritive matter under favourable conditions of moisture, temperature, aeration, etc.

It is highly probable that the spores and conidia of many of the fungi in the above lists can retain their vitality for months, perhaps years—the conidia of *Stysanus medius* (Sacc.), for instance, when transferred from a dried up culture to a fresh medium germinated after being dry for at least 12 months; when similarly tested, the conidia of *Periconia felina* (E. March.) had retained their vitality for at least 18 months, those of *Fusidium viride* (Grove) at least 15 months, while the spores of *Mucor circinelloides* (Van Tieghem) germinated after being dried up at least 2 years and 7 months, and the ascospores of *Chaetomium crispatum* (Fckl.) 2 years and 6 months. But it is most improbable that spores and conidia under salt marsh conditions retain their vitality long, since, unless they germinated almost immediately, they would yield to the activities of salt marsh bacteria, or be devoured by mites and Collembola or such-like soil fauna.

But it is hardly necessary to consider how long spores and conidia will retain their vitality, since one can postulate the continued reintroduction of fresh spores and conidia, blown or carried in from decaying

vegetation on the dunes near by, or from the extensive ill-drained moorland and peat-bog area bounding the salt marsh on the south, or from the uplands around.

And as for the common species such as *Torula allii* (Harz), *Penicillium hyphomycetis* (Sacc.), *Fusarium oxysporium* var. *resupinatum* (Sherb.), and others which occur in most soil samples, salt marsh conditions must be so favourable to their continued existence, that there can be little chance of that being dependent on their conidia possessing a prolonged period of vitality.

The condition in which fungi exist in the soil, whether as spores or as actively functioning mycelium, has been a subject of much discussion.

According to Waksman (20) and Brown (6), fungi in the soil are in an active mycelial condition—a view confirmed by McLennan (7) who states: "Fungi are present in the soil extensively, in fact practically entirely in the mycelial condition."

On the other hand, Winogradsky (17), employing the direct microscopic method, considers that fungi are present in the soil as spores, which only in the presence of organic material germinate and produce active mycelium.

The writer considers that fungi in the soil are active only in association with organic material, and since in most soils there is generally dead organic matter, and more or less satisfactory conditions as regards temperature, aeration and moisture prevail (for some fungi at least), there is no reason why fungus spores should not germinate and produce an active mycelium.

In a direct microscopic examination of the soil of this salt marsh, conidia and hyphae are rarely met with; however, if bits of dying organic matter (rootlets, leaves, etc.) from the soil are examined, hyphae can usually be found. Several times quite a good development of hyphae extending out into the soil around was found when decaying roots of *Glyceria* from soil samples taken 12 inches below the surface were examined.

But in the soil of this salt marsh, the conditions—a badly aerated tenacious clay, alkaline in reaction, a high water content due mainly to periodical inundations by salt water—are not those generally considered favourable to fungi; hence it was somewhat of a surprise to find fungi apparently able to tolerate conditions there.

Most soil fungi growing actively in culture generally produce quantities of conidia, yet in direct microscopic examination of the soil, conidia and hyphae too, as stated above, are rarely met with, and a conidium

attached to a hypha was not seen: this may be due to the activity of soil fauna, for the voracity of such organisms as mites and Collembola for conidia and hyphae in cultures is only too well known to the writer.

If dying or dead rootlets are well washed and rinsed repeatedly in sterilised distilled water and transferred to hanging drops, a copious development of hyphae soon follows, usually with abundant conidia: the nutriment on which the fungus is living is doubtless the organic tissue of the rootlets; but in the soil of such a salt marsh as this, nutrient solutions from the excrement of sheep and of birds must also be present in abundance, on which saprophytic fungi can thrive.

Both *Glyceria maritima* and *Armeria maritima* are mycorrhizal plants, in fact in the former the fungal infection extends throughout the plant but it is not obligate, since it is quite possible to grow *Glyceria* plants from seed in sterile soil free from mycorrhizal infection; plants from a salt marsh transferred to ordinary garden soil still continue to produce infected roots[1].

Fungal infection probably extends also throughout *Armeria* plants too, since hyphae are always present in leaf bases as well as in roots.

It is probable that some of the mycorrhizal fungi are identical with those in the above list; in fact, it is significant that during an investigation of the anatomy of *Glyceria* by Mason (16), the writer saw a preparation of a root containing a fungus which appeared identical with *Monilia pruinosa*—a fungus in the above list. Further, from the rootlets of *Glyceria* endophytic fungi (perhaps mycorrhizal), which were identified as *Stachylidium cyclosporum* (Grove) and *Cladosporium herbarium* (Link.), appeared in hanging drop cultures: the latter fungus may be identical with *C. lignicolum* (Corda), a fungus included in the above list.

GLYCERIA AND *ARMERIA* IN RELATION TO SALT MARSH SOIL FUNGI.

It is well known that saprophytic fungi bring about a natural decay of plant tissues, in fact fungi seem able to decompose most constituents of plants (celluloses, hemicelluloses, sugars, pectins, pentosans, etc.) except lignins, but even the latter yield to the activities of some of the higher fungi and perhaps a few of the Fungi Imperfecti. Several Actinomycetes are also reported to break down lignins and the same may be said of one or two bacteria.

The efficiency of *Glyceria maritima* as a marsh builder is doubtless bound up with the fact that most of the aforementioned soil fungi are unable to decompose lignins, and even though several of the above species

[1] I am indebted to Miss M. Going, B.Sc., for carrying out these experiments.

have been proved to be capable of decomposing cellulose (*Trichoderma Köningi*, *Penicillium expansum*, *P. decumbens*, *P. claviforme*, *Aspergillus fumigatus*, *A. glaucus*, *A. flavus*, *Acrostalagmus cinnabarinus*) and most of the others belong to genera known to include species which decompose cellulose, yet the waterlogged condition of the soil (most of the Glycerietum is covered by every tide) and its lack of aeration must tend to retard their activities.

Glyceria maritima, which is a most effective silt binder when colonising bare ground, produces creeping shoots from which extend quantities of long fine roots and rootlets: later the plants take on a tufted habit and continue producing innumerable fine roots, which may extend a depth of 2 feet or more. The effectiveness of such a plant as a silt binder is seen when trying to free it from silt for investigation purposes—a most tedious operation.

Sections through the rootlets of *Glyceria maritima* reveal a cortex with a fair development of intercellular spaces, and a mycorrhizal infection. This cortex becomes gradually disintegrated owing to fungal action, leaving a stele surrounded by cork. The stele is very compact, and consists almost entirely of lignified tissue. Sections through the rhizomes show a similar structure with a strongly developed zone of pericycle fibres, but only slight mycorrhizal infection.

In some parts of the marsh during a stormy period erosions take place, when dense cottony masses of *Glyceria* are left exposed. In such masses dead roots and rhizomes are found in various stages of decay, due to the activity of both fungi and bacteria, but for the most part the stele of the root and the pericycle of the rhizome—the lignified parts—are intact.

There is little doubt that this resistance of *Glyceria* to decay is in part due to the salt marsh soil fungi being chiefly of those species unable to decompose lignin: even if many lignin-decomposing fungi were present, the waterlogged condition of the soil and its lack of aeration would doubtless retard their activity; however, it must be remembered the cortex is quite well aerated, and any fungi which enter are under better conditions for carrying on their activities than in the stiff, badly aerated soil outside.

The marsh teems with soil bacteria, and it is highly probable that in the decomposition of cellulose, pentosans, etc., they take an active part, the waterlogged condition of the soil not checking their activities as in the case of fungi.

Now *Armeria maritima* is not nearly so effective a salt marsh builder

as *Glyceria maritima*. *Armeria* does not grow so rapidly as *Glyceria*, and for this reason would not be so successful as the latter in a region where accretion is somewhat rapid, and "accretion is much more rapid in the Glycerietum than in the Armerietum[1]": further, *Armeria* is not so well equipped to resist attacks of fungi and bacteria.

In the Armerietum basin-like depressions—the so-called pans—are numerous where salt water lies for a considerable time. Now where *Armeria* grows at the margin of a pan an escarpment is seen, usually considerably undermined, thus demonstrating the ineffectiveness of *Armeria* as a silt binder: on the other hand, where *Glyceria* is found at the margin of a pan there is usually a sloping shore.

Sections through the rhizome and roots of *Armeria* show that the total amount of lignified tissue is small, there being a conspicuous absence of mechanical tissue of the nature of sclerenchyma, the only lignified tissue being the xylem and of that there is only a small quantity.

Further, as regards aeration, in young roots a good development of small air spaces exists, and in old roots with secondary thickening there are large triangular air spaces. The rhizomes too are well supplied with air spaces.

The Armerietum, although submerged during all spring tides and perhaps during some neap tides, gets more drainage than the Glycerietum, and so as a medium for the activities of fungi is a degree or so better. Thus *Armeria*, as a plant having little lignified tissue and possessing large air spaces, succumbs to the activities of fungi far more readily than *Glyceria*, and, at a depth of a foot, it is usual to find the tap roots of *Armeria* little more than hollow tubes of cork, the whole interiors having been removed by the activities of fungi and bacteria. Further, after erosion has taken place during very high tides, escarpments in the Armerietum, as one would expect, show no great tangle of roots and rhizomes, as is the case of the more lignified plant *Glyceria*.

I have not thought it necessary to give an historical survey of the work done by various investigators on Soil Fungi, since excellent reviews of the subject have been given by Jensen (11), Waksman (3), Coleman (18), Brierley (4) and others.

In conclusion, I should like to express my thanks to Mr W. B. Grove, M.A., for critically examining certain fungi, and to Miss E. M. Wakefield for confirming my opinion that certain fungi have not been recorded before for the British Isles.

[1] A statement from Prof. Yapp.

Summary.

In this investigation of the fungi of the soil of the Dovey Salt Marshes (Ynyslas) 48 fungi were isolated.

Twelve of the species found do not appear to have been recorded before for the British Isles.

The area investigated is a badly aerated, stiff, tenacious clay, alkaline in reaction (pH 8), with a high water content, due mainly to periodical inundations by tidal salt water.

Method of investigation consisted in taking samples of soil from a depth of $1\frac{1}{2}$ and $3\frac{1}{2}$ inches and either planting portions of this directly on to specially prepared media, or first shaking up a portion in water and then inoculating the prepared media with some of the suspension.

Three fungi—*Torula allii*, *Penicillium hyphomycetis* and *Fusarium oxysporium* var. *resupinatum*—were almost invariably present in every sample of soil: almost equally common were *Trichoderma lignorum*, *T. Köningi*, *Hormodendron cladosporoides*, *Mucor circinelloides* and *Periconia felina*.

Most of the fungi found are species found above ground as saprophytes, and may have been introduced into the soil by drainage, etc.

The writer considers fungi are active only in association with organic material.

The vegetation covering this marsh shows marked zonation: soil samples were chiefly taken from the Glycerietum and Armerietum.

The same species of fungi were common to the two associations.

Glyceria maritima is a most effective silt binder, because the stele of its rhizomes and roots consists almost entirely of lignified tissue which does not yield to the activities of the soil fungi; *Armeria maritima* is not so effective a silt binder because the stele of its rhizomes and roots contains very little lignified tissue—at a depth of a foot, the tap roots of *Armeria* are little more than hollow tubes, the interiors having been removed by fungi and bacteria.

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THE PRINCIPLES OF BIOLOGICAL CONTROL

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CONTENTS.

I. Introduction.

In spite of the remarkable and rapid development of practical operations in biological control, the fundamental principles underlying the work have received, up to the present, relatively little attention. It is obvious, however, that, without careful theoretical and experimental studies of the action of the organisms it is desired to utilise in the control of pests, the result of practical experiments in this direction can never be properly understood; and since little or nothing of a definite character has been learned from what has been done, it is impossible to plan future operations to any better advantage. A general survey of the subject is therefore desirable, the more so since the idea of biological control has now become fashionable and is tending to degenerate into a kind of superstition or fad.

In the present paper, I propose to consider briefly the nature of biological control and its relation to natural control in general, the nature and practical value of biotic controlling factors of various types,

the objects to which biological control can be applied, the situation in which the method can be utilised and the results which may be expected from it. To those familiar with the subject a good deal of the discussion may seem elementary; but it is nevertheless fundamental and, I believe, necessary if a sound and reasonable view of the subject is to be obtained.

II. The nature of biological control.

The word *control* may evidently have several different meanings. It is used in this paper to designate a check on the increase of an organism, or, in other words, a diminution of its rate of multiplication, brought about by any cause, or combination of causes, whatever. When the diminution of the reproductive rate is such that there is no further increase, the population remaining numerically constant from generation to generation, the organism will be said to be *completely controlled*. When it increases at any rate between the point of complete control and the rate of increase attained when all of the progeny an individual can produce under optimum conditions, attain maturity and reproduce in every generation, it will be said to be *partially controlled*. Used in this way, these terms have, of course, no definite connection with the question of *economic control*. Whether an insect pest is in control from the standpoint of the economist depends upon whether the injury resulting from its presence causes a financially measurable loss. This depends, in turn, other things being equal, upon what we might term very roughly, the number of individuals of the pest per unit of area. A pest may be thus in economic control, although it is increasing and spreading, or it may cause severe economic losses and yet be completely controlled in the sense that its population remains practically stable from generation to generation. Nevertheless, although during the initial period of its increase, a pest may not cause any appreciable economic damage, it will ultimately become injurious unless its increase is checked; and while an organism may still cause economic damage, even though it is, technically speaking, in complete control, still the prevention of damage is much easier to bring about when the population is stable than when it is on the increase. The notions of technical and practical control, while not interchangeable, are thus closely connected. For the purpose of the present argument, the word will be used, mainly, in the technical sense.

By *natural control* we mean the reduction in the reproductive rate of a species effected by *natural* as opposed to *artificial* factors or, to put it in another way, the control which occurs in the natural environment as opposed to an environment artificially created by man.

By *artificial control* we mean the reduction in the rate of multiplication effected through human intervention. It must, however, be noted that control of this type may be brought about either through manoeuvres specially designed to reduce the numbers of the pest, or may occur accidentally, during the course of the agricultural operations normally carried out in connection with the affected animals or plants. Thus, as we have been informed by M. Regnier, the codling moth in certain parts of Normandy is of very little importance, because the orchards are used as pastures for cattle, which eat the wormy fruit as soon as it falls to the ground. Similarly, the method of cropping lucerne in Southern Italy, has, as an accidental result, the removal of the eggs of the weevil, *Phytonomus posticus*, from the fields.

By *biological control* we mean the reduction of the rate of multiplication of an organism effected through the agency of other organisms as distinct from non-living factors. The effect exerted by organisms upon each other may be direct or indirect. Every organism has an effect upon its fellows through its general action upon the common environment. The effect of such indirect action upon the formation and development of biological associations is undoubtedly enormous. During the course of its ordinary existence and in the pursuit of its own ends, the organism prepares an environment in which certain species will flourish but from which others will be excluded. In so far as relations of this type between organisms produce a reduction in the reproductive rate, they constitute *indirect biological control*. They need not be considered in detail because their complex and essentially contingent character renders it impossible in most cases to utilise them in practical work.

Direct biological control results from the direct specific actions of certain definite organisms upon certain others which they habitually injure or destroy. Any organism which habitually injures or destroys other organisms may be considered as an agent of biological control. Since most animals and many protozoa, bacteria and fungi, as well as certain of the higher plants, prey and develop at the expense of living organisms, the number of such controlling agents is enormous.

This classification of controlling agencies is sufficient for the purpose of this paper, but it is of course inadequate as applied to the course of events in Nature, where intermediates of all degrees exist. For example, in the regions which chiefly interest the economic biologist, human intervention has operated through so long a period and has so greatly changed the character of the fauna and flora, that it is practically impossible to pick out any area in which conditions may be considered

absolutely "natural." Furthermore, it is difficult to draw any practical distinctions between physical factors of control, considered independently, and these same factors as produced by the various biotic factors, or to decide whether certain domesticated or semi-domesticated animals and plants are to be classified as natural or artificial factors.

It is equally difficult to make any general statement as to the value of various types of controlling factors. As has been pointed out in a preceding paper[1], the natural control of organisms is primarily due, not to any complex cosmic mechanisms or regulatory factors, but rather to the intrinsic limitations of the organisms themselves. Every organism, because of its specific characteristics, has specific needs or, in other words, it requires, for the complete realisation of all its powers, a certain specific environment, in the absence of which its activity declines. One of the characteristics of the optimum environment is, of course, the absence of the parasitic and predaceous species to which the organism concerned is attractive; but the environment must also possess certain definite physical characteristics. Thus, either the presence of one or more species of inimical organisms or the departure of one or more of the physical factors from the optimum intensity, or a combination of the two, may produce partial or complete control.

Control, considered from the standpoint of the organism, is due essentially to inimical influences and can be most easily understood by remembering that in this case, as in all others, evil is simply the absence of good. It is thus, so to speak, founded upon a negation, the negation of *what is good for the organism*—and therefore cannot be brought within the compass of exact definition. The fact is that the causes of the natural control of a given organism differ from point to point over the area it inhabits and from season to season. This is true of insects as of other organisms. In one area the rarity of an insect pest may be due to the peculiar distribution of the host plant; in another to the presence of parasitic enemies; and in a third, to an unfavourable climate, though frequently it will be due to a combination of several such factors, each of which contributes to the maintenance of control.

Whether biotic or physical factors predominate in the control of a given species in a given area, investigation alone can determine. Dr L. O. Howard once stated, in discussing the value of birds in relation to injurious insects, that if all the birds vanished from the earth, the status of insect pests would change very little, because their insect parasites and predators would soon increase sufficiently to maintain control. But

[1] Thompson, W. R., "On natural control," *Parasitology*, 1929.

when we consider the matter broadly we may go even further and say that, if all the parasitic and predaceous insects disappeared, this would not mean the complete release of their hosts from control. Certain changes in the composition of the flora and fauna, which might have temporary economic consequences of a very serious nature, would probably occur; the relative populations of individuals of certain species would alter, but the natural diversity of environments would be quite sufficient to prevent any of the species concerned from increasing indefinitely.

Since the complex of environmental conditions responsible for the control of insect pests may vary in composition, both quantitatively and qualitatively, in different parts of the area inhabited by the species, we may conclude that the measures necessary to re-establish control, in the case of an outbreak, will also vary in different times and places. It would, therefore, be extremely unwise, especially when we have to deal with all of the major pests of a large area, to rely exclusively upon the methods of biological control for the solution of our problems. The method has many advantages, but it is not possible to employ it successfully against all pests, nor is it by any means the only weapon in the arsenal of the economic entomologist.

III. The factors of biological control.

As we have already said, most animals and many protozoa, bacteria and fungi, as well as certain of the higher plants, prey upon and develop at the expense of other living organisms, of which they are, therefore, controlling agents, so that the number of biotic controlling factors is enormous.

The biotic controlling factors of insect pests, with which we are chiefly concerned in this paper, may be classified roughly under the following main headings:

(1) Disease-producing organisms, including protozoa, bacteria, fungi and ultramicroscopic organisms.

(2) Invertebrate parasites and predators, including nematode worms, insects, myriapods, spiders and acarina.

(3) Vertebrate predators.

In this section the comparative value of these factors in practical work will be briefly considered.

(1) *Pathogenic organisms.*

The organisms of the first group mentioned, producing the conditions ordinarily called disease, are extremely varied in structure and mode of life. A considerable number, especially among the protozoa, are only feebly pathogenic. All of them are organisms of relatively simple stucture and very small dimensions, which multiply for a long series of generations within the body of the host before causing death, an enormous number of individuals being necessary to produce a lethal effect. Unlike other biotic factors, the lethal power of the disease-producing organisms in relation to a given host, is variable and often increased by their passage through that host.

Since the disease-producing organisms are extremely minute and relatively homogeneous, or at least simple in structure, their adaptive powers would appear to be necessarily limited, so that they can subsist, reproduce and pass to new hosts only under certain very special conditions. When hosts are abundant and closely massed together, and meteorological conditions are favourable, pathogenic organisms often multiply and spread with great rapidity, producing tremendous epidemics. It is, however, extremely difficult to engender these epidemics artificially. The organisms which cause them are usually so widely dis tributed in Nature, that little is gained by disseminating them. If conditions are favourable, epidemics occur naturally; if they are not, the distribution of the causative organism does not produce them. Many attempts to utilise them practically have been made by entomologists and others; but no decisive results have ever been obtained. The last word, perhaps, has not been said in regard to this subject, particularly in tropical areas, and an investigation of the genesis and conditions of epidemics among insect pests is highly desirable, but at the present there seems little hope of utilising the pathogenic enemies of insects in practical work[1].

(2) *Invertebrate predators and parasites.*

The organisms of the second group include, in the first place, certain of the nematode worms, of which the most important belong to the family Mermithidae. These worms sometimes infest a large percentage of individuals of certain species, particularly among the Orthoptera, in which they often produce a form of parasitic castration and prevent

[1] Paillot, A., "Les Microorganismes parasites des insectes," *Ann. des Epiphyties*, T. 2, Paris, 1916.

reproduction, but though their potential reproductive powers are great, their controlling value is, at present, uncertain, though an attempt to utilise them against grasshoppers is now being made by Cobb and his associates in America.

The most numerous enemies of insects are the parasitic and predaceous species of the same group. The vast majority of the parasitic forms belong to the Diptera and Hymenoptera, though some species with this habit are found in other orders. Predaceous entomophaga occur in many groups.

The relative value of the parasitic and predaceous enemies of insects has been discussed at some length in a previous paper[1], and need not be considered further at present. It may, however, be noted that the entomophagous insects, whether parasitic or predaceous, are, for the most part, comparable in dimensions and reproductive powers to the species on which they prey. If adaptability is a function of structure they must be in general as adaptable as their hosts and seem, in fact, to be equally general in their distribution. They are, therefore, the most effective and dependable of the organic checks to insect increase, being more regular in their action (or, to put it more exactly, less affected by slight environmental variations) than the pathogenic micro-organisms and are more rapid breeders than the vertebrates. By far the greater part of the work carried on in the field of biological control, therefore, relates to the entomophagous insects and it is with the members of this group that the most striking successes have been obtained.

(3) *Vertebrate predators.*

The question of the predaceous vertebrates is more difficult. Animals belonging to this group, and birds in particular, undoubtedly destroy great numbers of injurious insects, which, like other predators, they often kill before the hosts have finished feeding, thus providing to some extent the same relief as is obtained by methods of mechanical control; but their reproductive power is very small as compared with that of insects, and their rate of increase, on the whole, much slower. The number of individuals of any given species is also relatively small in comparison with those of insects, and there is no reason to suppose that it varies primarily in function of the supply of insect food, which fluctuates so rapidly that it is impossible for vertebrates to profit by a temporary abundance of it, excepting to a very limited extent. In order to make clear the significance of this point, let us imagine a case where we have

[1] Thompson, W. R., *Bull. Ent. Research*, XIX, Pt 4, March 1929, pp. 343–50.

ten insectivorous birds working on a population of 10,000 insects, of which they destroy 100 each, thus accounting for a total of 10 per cent. Suppose that, owing to some unusual conditions, the insect begins to increase at a rate of four-fold per annum. Unless the absence of food has actually been limiting the rate of increase of the birds, the increase of the insect considered will not affect their numbers. The *number* of insects they destroy will, therefore, remain constant and the *percentage* they destroy will fall steadily, as shown in the following table:

	Percentage destroyed	No. of hosts
G. I	10	10,000
G. II	2·8	36,000
G. III	0·7	140,000
G. IV	0·18	546,000

It has, of course, been maintained that, in such cases, the abundance of food material attracts large numbers of birds from surrounding areas. There is, however, little evidence that outbreaks of major pests are often controlled in this way, and, on the other hand, it must be noted that the concentration of birds in one area means their disappearance from another, in which the insects controlled by them would be left free to increase, so that what was gained at one point would be lost at another.

It has been claimed in recent years that bats can be employed to keep down many insect pests, particularly those which are nocturnal fliers in the adult stage; but further investigations have failed to support these claims[1]. We must therefore conclude that, although vertebrates, and particularly such mobile forms as birds, bats and lizards, certainly exert some influence as biotic factors of control, their regulatory powers in relation to insect outbreaks are slight, in spite of the fact that they sometimes congregate in areas of high infestation[2].

At all events, there is at present no reason for supposing that the *introduction* of native vertebrate enemies of imported food pests would have any noticeable effect in checking their increase and spread. The protection of indigenous birds, especially the insect feeding species, is no doubt desirable on general grounds. But in spite of the claims that

[1] Nelson, E. W., "Bats in relation to the production of guano and the destruction of insects," *U.S. Dept. Agric. Dept. Bull.* 1395, Washington, D.C., 1926.

[2] On the question of the value of vertebrates as controlling agents, see: Fiske, W. F., *Parasites of the gypsy and brown-tail moths introduced into Massachusetts*, Boston, Mass., 1910; Howard, L. O. and Fiske, W. F., *Bull.* 91, *U.S. Dept. Agric., Bur. of Ent.* 1911; Strickland, E. H., "Can birds hold injurious insects in check?" *Scient. Monthly*, XXVI, 48–56, Jan. 1928; Elton, C., *Animal Ecology*, London, 1927.

have been made, we have at present no real proof that they are factors of major importance in insect control. The majority of arguments so far advanced are extremely unconvincing and have in general relatively little bearing upon the point at issue.

IV. The objects of biological control.

By "objects" of biological control, we mean the noxious organisms to which the methods of biological control may be applied—whose control may be effected, or, at least, attempted by utilising other organisms which prey upon them. As almost every living creature injurious to man, has plant or animal enemies, the number of experiments it is possible to make in this direction is almost limitless. However, as we are here writing for entomologists, we shall consider only the two entomological branches of the work; viz. biological control of *noxious insects* by *entomophagous insects* and biological control of *noxious plants* by *phytophagous insects*, with a view, more especially, to comparing the susceptibility of insects and plants to insect attack. Such a comparison seems the more necessary, because the idea of controlling noxious weeds by means of phytophagous insects, originated apparently by Perkins and Koebele in relation to *Lantana* in Hawaii[1], and adopted some time later by the Australian authorities against prickly pear[2], has in recent years become increasingly popular.

The difference between the problems of the control of insect pests and plant pests by insect parasites and predators depends essentially upon the fact that the insect, like the majority of animals, is a highly individualised and highly centralised organism, with a precisely defined form, while the plant is a very feebly individualised and relatively amorphous organism. With this difference are correlated many others which it is superfluous to mention, and one difference, which is, from our present standpoint, of primary importance: *difference in susceptibility to injury.*

Because of its highly individualised and closely co-ordinated structure, the animal, though it has greater powers of action than the plant, is more dependent for its continued existence upon the well-being of each one of its several parts. The animal is highly mobile, but, if one of the outgrowths by which it moves is amputated, its chance of sur-

[1] Perkins, R. L. and Swezey, O. H., "The introduction into Hawaii of insects that attack *Lantana*," *Hawaiian Sugar Planters' Ass. Bull.* 16, 1924.

[2] Dodd, A. P., "The biological control of prickly pear," *Journ. Coun. Sci. Ind. Res.* I, No. 1, Melbourne, 1927.

vival is greatly diminished; the plant is hardly mobile at all, but local injury and removal of even a considerable portion of its ill-defined form affect it little. In other words, the host of the phytophagous insect is more difficult to injure than that of the entomophagous insect.

Furthermore, the phytophagous insect is usually of relatively small dimensions as compared with its host, whereas the entomophagous insect often equals or surpasses the host in size, so that the destructive capacity of the latter in relation to its host is naturally greater than that of the former. The consequence of this is that the action of phytophagous insects in relation to the individual host plant must be a great deal more intense than that of the entomophagous insect in relation to the individual host insect, if they are to exert any perceptible effect as controlling agents. Parasitic insects, in general, kill all the individuals on which they feed, and though in gregarious species a number actually combine to produce the death of a single host, even one of these is usually sufficient to produce death. Predaceous insects, in many instances, kill more individuals than they devour, taking from each only a small part of the food material it contains. The phytophagous insect, in general, very seldom kills the host on which it feeds, the combined efforts of an exceptionally large number of individuals being necessary to produce death.

A general study of the effect of phytophagous insects on vegetation has never been made and is greatly needed. However, any biologist who has observed the work of plant-feeding species in Nature will admit that obvious signs of severe injury are uncommon, while the total destruction of plants is extremely rare.

It is quite true that the damage resulting from the attacks of injurious insects on economic plants is frequently considered to be serious, but in this case the importance of the injury is measured chiefly by financial loss, or, in other words, in the depreciation in the marketable value of the crop. An injury which is relatively slight so far as the plant is considered may be of considerable importance financially; and except in the case of such exceptional and devastating plagues as locusts, army worms and so forth, it is very seldom that insect injury is serious in the former sense.

In order that the action of phytophagous insects may produce control, the plants attacked must be so severely injured that they are unable to survive or, at all events, unable to reproduce or spread. Now, excepting perhaps in the case of certain annuals, even complete defoliation is not necessarily fatal, while in the case of plants with bulbs, tap roots, or

underground runners, as well as those of shrubs or trees, several defoliations may be required to cause death. Furthermore, although insects which feed upon both the vegetative and sexual reproductive systems of plants are very common and at times rather abundant, we have at present little evidence to show that they are of great importance in the limiting of the increase and spread of their hosts.

The whole practice of biological control is, of course, based upon the idea that when a phytophagous insect migrates to a region in which its native parasites do not exist, its powers of multiplication and destructive capacity increase; and if this is true of the phytophagous insects attacking noxious plants, it ought also to be true for those attacking useful plants; but, as we have already said, the injury produced in such cases, though often economically serious, is generally below the level of what is required for the *control* of vegetation.

On general grounds, therefore, phytophagous insects seem to be less promising than entomophagous insects, as agents of biological control. They are also more dangerous. It is true that entomophagous insects do not always confine their attacks to the pest against which we try to use them; but more often than not, the other insects they attack are themselves injurious, attacking plants which are useful to man. In some cases, no doubt, introduced parasites or predators may attack insects injurious to weeds, but, as we have already said, there is at present no definite proof that the rôle of such insects in Nature is of any real importance. When a phytophagous insect, introduced to control a weed, moves to a useful plant, the results may be more serious because, in this case, injury quite insufficient to effect control in the technical sense may render the crop absolutely worthless in the market.

The results obtained in Australia by the importation of the insects attacking prickly pear and in Hawaii by those attacking *Lantana*, are certainly promising, and so far these experiments seem to have had no serious consequences; but, in view of the dangers and the difficulties of the problem, it is necessary to be extremely cautious about the extension of this type of work to other plants, to make no introductions until exhaustive tests have been carried out, and to investigate fully both the general question of the rôle of the insect enemies of weeds and the problem of the natural control of these plants in their native homes.

V. THE OCCASIONS FOR BIOLOGICAL CONTROL.

The fundamental requirement for the biological control of a pest, is, of course, the existence, either in the area inhabited by it, or elsewhere, of biotic controlling factors—parasites, predators or pathogenic organisms—which are attracted by it, will prey upon it, given the opportunity, and are capable of multiplying at its expense, in areas in which it is injurious. The work undertaken may have as its object either:

(1) the intensification of the action of natural enemies already present in the area; or

(2) the introduction of natural enemies absent from the area.

In the second case the natural enemies may be either:

(*a*) species normally or occasionally present in the area but temporarily absent from it; or

(*b*) species never before present in the area.

In the latter case, the parasites or predators introduced may be either:

(i) species of which a known host already exists in the area into which they are brought; or

(ii) species of which no known host exists in this area.

In actual practice the work against a given pest may, of course, entail a combination of several of these methods. We may now consider these various possibilities in order.

(1) *Intensification of the action of natural enemies present in the area.*

Suggestions for intensification of the action of the native enemies of insect pests were amongst the earliest made by naturalists in respect to biological control. Kirby and Spence, in 1816, pointed out the importance of the common English ladybird in destroying the hop aphis, and wrote that if it were possible to induce them to increase at will, hot houses could be cleared of aphids and hop crops rendered much more certain than they ordinarily are.

Attempts of this kind may, as Myers[1] has already pointed out, be considered, in a certain sense, as efforts to extend the process of domestication to beneficial insects. Practical experiments along these lines have been made by gardeners and others for a long time past and are still being continued in various parts of the world[2]. It is impossible to

[1] Myers, J. G., "Biological control," *Trop. Agric.* VI, No. 6, pp. 163–5, Trinidad, 1929.

[2] See: Howard and Fiske, *Bull.* 91, *U.S. Dept. Agric. Bur. Ent.*, for a partial account of some of the past work of this kind.

discuss them in this paper in which only the general aspect of the question will be considered.

Generally speaking, attempts to intensify the action of indigenous parasites or predators consist either in a reduction in the numbers of the host or an increase in the numbers of its parasite or predator, or in the simultaneous removal of the hosts and addition of the natural enemies. The effect produced in all these cases is, of course, simply a readjustment of the population ratio between the noxious and beneficial insects, in favour of the latter, but, in order to facilitate the discussion of the subject, it seems best to distinguish between the attempt to reduce the host population and to attempt to increase the parasite population and consider them separately.

The attempt to encourage parasites and predators by an *artificial reduction* in the numbers of their hosts may be considered to have two principal effects, the first of them being a reduction in the damage caused by the host insect, and the second being the alteration, in favour of the parasite, of the numerical ratio between host and parasite populations.

The practical value of the destruction of the host insect depends principally upon:

(1) The proportion between the number destroyed and the population present in the area treated.

(2) The proportion of the host population for the destruction of which the measures adopted are essential.

(3) The moment at which the destruction is effected, and, in particular, whether the reduction in the numbers of the host occurs before or after the destructive phase in its life history.

A considerable reduction in the population of the pest must be effected in order to produce results of economic significance. A slight alteration will have, at best, only slight effects, and it is not likely to transform an economic failure into an economic success and make the difference between a worthless and a marketable crop.

The ordinary methods of mechanical control, such as spraying, fumigation and so on, can in many cases be relied upon to produce a reduction in the numbers of the pest, sufficient to allow of a crop which can be sold at a reasonable profit; but, as a general rule, these methods cannot be applied in such a way as to kill the pests without injuring their parasites. They do not alter the ratio between the population of the pest and that of its enemies and cannot therefore be said to encourage the latter.

On the other hand, methods of destruction designed as so to reduce the host population, but not that of the parasite, are, in general, laborious and expensive. The procedure usually followed is to collect the hosts, which are destroyed only after the emergence of the parasites, as in the West Indies, where the eggs of the sugar-cane borer are collected by gangs of labourers and preserved until after the issuance of *Trichogramma*, or, as in the measures suggested by Comstock and others[1] for the encouragement of the parasites of various injurious insects, by collecting chrysalids or pupae and keeping them in boxes covered with gauze, permitting the parasites to escape, but preventing the exit of the pest.

In order to estimate the difficulty of carrying out such methods efficiently, we may consider briefly a practical example.

A fairly heavy infestation of the European corn borer (*Pyrausta nubilalis* Hubn.) may contain over one million larvae of this species per acre. This will mean, as a rule, about the same number of egg masses. Suppose that in an infestation of this type we decide to collect egg masses which will be destroyed after the emergence of any parasites which happen to be present. If we estimate the time required to discover and collect an egg mass at one minute—which is certainly not excessive—the collection of 100,000 masses would require the labour of 20 men, working 8 hours a day for a period of 10 days, and although the number of egg masses collected would be numerically large and consequently impressive, the value of the work would really be very small, as only 10 per cent. of the population would have been destroyed.

Furthermore, a great deal of the effort exerted would be entirely valueless, since a large proportion of the individuals destroyed would have succumbed in any event before doing any perceptible damage. For example, in an average environment, from 80 to 90 per cent. of the larvae of *Pyrausta nubilalis* usually disappear shortly after hatching, or, at all events, during the very early stages. If we suppose that in the field in which 10 per cent. of the eggs are collected, this natural mortality would normally account for 90 per cent. of the larval population, the total mortality due to the work of the collectors and the natural environmental factors taken together will be:

$$[0{\cdot}1 + (1 - 0{\cdot}1)\,0{\cdot}9] = 0{\cdot}91,$$

or 91 per cent., and the increase in the death rate due to the collection of the eggs will only be 1 per cent. The reduction of the damage effected

[1] V. Howard and Fiske, *l.c.*

by the collection of hosts in such a case, which is not of an excessively unfavourable type, would be absolutely negligible.

As a matter of fact, the methods suggested for encouraging the work of parasites by the destruction of their hosts depend in many cases on the collection of individuals of the pest after its period of destructiveness is over. In such cases, there is, of course, no reduction in damage whatever. Therefore, unless the destruction of the host insects can be carried out as economically and efficiently as by the standard mechanical methods, no great immediate benefit is likely to be derived from the procedure.

However, the real object of the practice described, regarded as a method of biological control, is not an immediate reduction in damage, but a change in the numerical ratio of the reproducing populations of the pest and its enemies. We must now consider whether any appreciable benefit can be obtained in this way.

This question is a good deal more complex than it appears at first sight.

In the first place, as we have already shown, the change in the ratio between the population of the pest and its enemies actually effected by the collection and destruction of the former, may be much less important than the figures indicate, simply because many of the hosts would have succumbed in any event, long before the period when they are in a stage suitable for attack by parasites and predators. On the other hand, the fact that the parasites are allowed to emerge in captivity in no way increases their effectiveness, unless the measures taken ensure protection from dangerous hyperparasites, and may even be disadvantageous, because of the increased mortality resulting from the unnatural conditions obtaining in the emergence cages; so that, on the whole, the increase in the proportion of parasites to hosts, may be relatively slight, having regard to the work actually accomplished.

Suppose, however, that a definite change, advantageous to the parasite, in the ratio of parasite population to host population is obtained. In order to estimate the value of this change, it is convenient to consider separately cases in which we are dealing with stable populations and cases in which the populations are on the increase.

If the initial number of hosts be $= n$, the initial number of parasites be $= p$, the rate of multiplication of the host and parasite be respectively $= h$ and s, and both be considered for the purpose of the argument to be parthenogenetic female-producing species, then if we have

$$s = \frac{nh - ps}{n},$$

the ratio between host and parasite populations will remain stable from generation to generation, although the parasite reproduces at the expense of the host and both increase. If we have $s = 1$, our equation becomes

$$n(h-1) = p,$$

and in this event, not only the ratio between the number of parasites and hosts, but also the populations themselves will remain constant from generation to generation. Taking a case of this kind, let us suppose that in every generation 50 per cent. of the unparasitised hosts are destroyed after the emergence of the parasites. In such a case the course of events might be as follows:

G. I.	H. = 100 × 1·1 = 110	
	P. = 10 × 1·0 = 10	
		100, of which 50 per cent. are killed, leaving 50.
G. II.	H. = 50 × 1·1 = 55	
	P. = 10 × 1·0 = 10	
		45, of which 22·5 are killed.
G. III.	H. = 22·5 × 1·1 = 24·75	
	P. = 10 × 1·0 = 10	
		14·75, etc.
G. IV.	H. = 7·37 × 1·1 = 8·1	
	P. = 10 × 1·0 = 10	
		0·0

so that the numbers of the host are gradually diminished through the continuous effort.

Suppose, however, that with an exactly similar host, having no parasites at all, the same procedure was adopted. We should then have:

G. I.	100 × 1·1 =	110, of which 50 per cent. or 55 are destroyed;
G. II.	55 × 1·1 =	60·5, of which 30·25 are destroyed;
G. III.	30·25 × 1·1 =	33·3, approximately, of which 16·6 are destroyed;
G. IV.	16·6 × 1·1 =	18·2, of which 9·1 are destroyed;
G. V.	9·1 × 1·1 =	10·0, of which 5 are destroyed.

Thus, the elimination of 50 per cent. of the host would produce, in any event, a steady decrease in the population, though the decrease and the elimination would be slightly more rapid in the first instance because of the additional destruction effected by the parasite.

Suppose, however, that we had in each generation eliminated 60 per cent. of the pest, we should then have:

G. I.	100	$\times$ 1·1 =	110,	of which 110 $\times$ 0·4 = 44 remain;
G. II.	44	$\times$ 1·1 =	48·4,	of which 19·36 remain;
G. III.	19·36	$\times$ 1·1 =	21·3,	of which 8·50 remain;
G. IV.	8·5	$\times$ 1·1 =	9·3,	of which 3·7 remain;
G. V.	3·7	$\times$ 1·1 =	4·0,	of which 1·6 remain;
G. VI.	1·6	$\times$ 1·1 =	1·76,	of which 0·70 remain;

so that extermination would require little longer than when the parasite is present.

Taking now the case in which the population of the host is stable but that of the parasite is on the increase, let n = the initial number of hosts and p the initial number of parasites, with a reproductive rate = s. If there is no artificial elimination of hosts, the number t_1 of generations required for the extermination of the host will be:

$$t_1 = \frac{\log \left\{\frac{n(s-1)+ps}{ps}\right\}}{\log s}.$$

Suppose now we have an elimination of the hosts in the proportion of $(1 - w)$ in every generation, after the issuance of the parasite, so that w hosts escape ($w < 1$).

The time t_2 in generations, required for the elimination of the host, will then be

$$t_2 = \frac{\log \left\{\frac{n(s-w)+psw}{psw}\right\}}{\log \frac{s}{w}}.$$

The comparison of the values for t_1 and t_2 will give us an idea of the practical utility of the destruction of a part of the host population after the issuance of the parasites. Put n = 100,000, p = 10 and s = 4 and give to w the values of 0·9, 0·3 and 0·1, which means that in every generation we have 10 per cent., 50 per cent. and 90 per cent. of the host population destroyed. We then have:

w	1·0	0·9	0·5	0·1
t_1	6·4	—	—	—
t_2	6·4	6·1	4·6	3·1

Put $p = 1$, other values being as before. We have:

w	1	0·9	0·5	0·1
t_1	8·09	—	—	—
t_2	8·09	7·6	6·1	3·7

Thus in these cases, when 10 per cent. of the host population is destroyed in every generation, the time required for control is about 95 per cent. of that required when no work of this kind is done. When 90 per cent. of the population is destroyed, the time required for control is somewhat less than 50 per cent. of that required when no work is done; or, to put it in another way, ten times as much work in destruction of the hosts will not produce control ten times as rapidly (or in one-tenth of the time), but only twice as rapidly. The extra effort expended in such a case may therefore not give a proportionate return.

It must also be noted, that if the host has reached a condition of stability before the work began, the artificial rarefaction of its population may produce a more or less marked diminution in the average effective reproductive rate of the parasite, because an individual will have to cover a much larger area in order to deposit its quota of eggs. The greater the proportion of hosts destroyed, the more the effective reproductive rate of the average parasite will be reduced, other things being equal, and, consequently, the more the process of control will be retarded.

In the case just studied, the reproductive rate of the parasite is supposed to be four times as great as that of the host. In order to get an idea of the value of the operation under discussion, when the difference in reproductive rates is less marked, put $n = 100{,}000$, $p = 1$, $w = 0{\cdot}5$ and $s = 1{\cdot}5$, 2, 3, and 4, we then have:

s	1·5	2	3	4
t_1	25	15	10	8
t_2	10	8	6	5

From these figures we may conclude that, other things being equal, the value of attempts to accelerate the process of control by the destruction of the host population is most marked when the difference in the reproductive rates of the host and its natural enemies is slight, which is a point in favour of the method, as marked differences in reproductive rates are probably unusual in Nature. However, even in these cases it remains true that beyond a certain point an altogether disproportionate effort is required, in order to produce a definite decrease in the time taken for control.

The last case to be considered is that in which the populations of both parasite and host are increasing. A case of this type has been studied in some detail in another paper[1], dealing with effects of methods of mechanical control on the progress of introduced parasites of insect pests, where it was shown that if such methods produce any marked destruction of parasites as compared to hosts, the time required for control may be considerably increased, but that the effect of favouring the parasite is much less marked and produces only a relatively slight reduction in the time necessary for control.

On the whole, therefore, we are obliged to conclude that methods designed with a view to the destruction of unparasitised and the preservation of parasitised hosts are of rather uncertain value and not likely, in most cases, to produce results proportionate to the time and labour expended. In some cases, as we have seen, the employment of such methods may produce a gradual rise in the percentage of parasitism in the field, which in practical work might give the impression that the parasites are becoming more efficient. It is, however, clear that such methods *in no way help the natural enemies present.* Unless they are prevented from doing so by the decrease in the numbers of their hosts, the parasites and predators will continue to deposit the same *number of eggs* and kill the same *number of hosts* as under normal conditions. The idea that in cases of this kind the beneficial insects present are *encouraged* in any way or indeed that this method has anything to do with *biological control,* is a pure illusion. The process is simply a special kind of *mechanical control.*

The attempt to reduce damage by noxious plants or animals by *artificially increasing* the population of their parasites or predators, normally present in the area considered, may be quite properly placed among the methods of biological control.

The object of attempts of this kind may be either:

(*a*) The *immediate* reduction of damage; or

(*b*) The acceleration of the multiplication of the beneficial insect so as to diminish the time necessary for it to exert its full effect.

As we have already pointed out, reduction of damage may be effected either by the destruction of individuals of the injurious species before they have begun their attack—as in the egg stage in the case of species with injurious larvae—or by the destruction of individuals after the injurious phase, to such an extent as to affect adversely the production of offspring in the following generation.

[1] Thompson, W. R., *Bull. Ent. Res.* XVIII, 13–16, 1927–8.

Since the reduction of damage is necessary in order to produce a profitable crop, it is evident that the effect previously exerted by the beneficial insects studied is insufficient.

In the case of beneficial species only recently introduced, this is often due simply to the fact that the initial number of individuals colonised was small, and that multiplication for many generations is necessary to bring the species up to the maximum population capable of existing in the area studied. In the case of indigenous species, on the other hand, it usually means that the *average* or *normal* effect exerted by the beneficial species is inadequate and, consequently, that the average and normal conditions in the area, keep its population permanently below the point where it is economically efficient.

Now, whether we are dealing with introduced or indigenous species, and whether we hope to reduce damage by preventing individuals from reaching an injurious stage or by lowering the rate of reproduction, the one thing essential to success is the production of a really considerable increase in the population of the beneficial species considered, in the field. All that has been said in regard to the destruction of the host population applies with equal force to additions to the population of parasites or predators. Even though the percentage of parasitism is quite small, the actual numbers of parasites present in a relatively limited area severely infested by the host species, may be very large. Thus, a moderate infestation of aphids on certain types of plants might contain a population of several millions of individuals per acre; and, consequently, a parasite population of tens of thousands or even hundreds of thousands per acre. In such a case, in order to produce a measurable effect in an area of any considerable size—as for example, a 10-acre field—it would be necessary to liberate a very large number of parasites. The addition of a few thousand individuals in such an area would, in all probability, have no perceptible effect. For example, suppose the population of the 10-acre field comprised 20,000,000 individuals, which is well within the bounds of possibility for many injurious species—and that 10 per cent. of these were parasitised and killed before reaching a destructive phase in their life history. In such a field the addition of 10,000 parasites would mean only an increase of one-twentieth of 1 per cent. in the percentage of parasitism and could have absolutely no economic significance, although the expenditure involved in producing 10,000 parasites might increase the cost of production considerably.

The attempt to accelerate the progress of an introduced parasite by

liberating additional colonies year after year, following the establishment of the species, may have equally little effect.

Let us suppose, in the first place, that the reproductive rates of host and parasites are equal. In this case, as has been shown in previous papers (using the symbols employed in preceding pages), the time t, in generations required for control, will be

$$t = \frac{n}{p}.$$

In such a case the length of time required for the increase of a beneficial species to the point where it exerts its maximum effect depends directly, other things being equal, on the numbers of parasites liberated. A colony of 100 parasites will control the host in one-tenth of the time required by a colony of 10 parasites. Suppose, however, that we have 10,000 hosts and 1000 parasites, with equal reproductive rates. In this case the host will be exterminated in 10 generations. If now, after the first generation, we liberate an additional 1000 parasites in every generation, the acceleration of the process of control, though definite, will not be as great as one might expect. The liberation of an additional 1000 will result in control in seven generations, but even when the liberations are continued until 5000 extra individuals have been released, six generations will still be required for control; the effect produced by the liberation of the third, fourth, fifth and sixth thousands being practically negligible, though the cost of operations might be greatly increased by the collection of this extra material.

When the reproductive rate of the parasite is superior to that of the host, the time t, in generations, required for control is

$$t = \frac{\log\left\{\dfrac{n\alpha - n + p\alpha}{p\alpha}\right\}}{\log \alpha},$$

when we have $$s = \alpha h \text{ and } \alpha > 1.$$

In such a case, the length of time required for the increase of a parasite to a point where it exerts its maximum effect, is relatively little affected by variations in the size of its population as compared to that of the host.

Thus put $n = 100{,}000$, $\alpha = 1{\cdot}5$ and $p = 1000$, 5000 and 10,000. We obtain for the value of t, 8·7, 5·0 and 3·5 respectively. Even though the parasite population is increased ten-fold, the time necessary for control is still more than one-third of that originally required. If now, taking the first case, we liberate after the first generation 1000 additional parasites in every generation, the acceleration of the process of

control will be extremely slight. The liberation of seven additional thousands will reduce the time required for control by less than a single generation. In such a case the expenditure required to make additional introductions, after the establishment of the parasite, would be almost completely wasted. The more rapid the increase of the introduced parasite as compared with that of the host, the less will subsequent introductions affect the progress of events.

The importance of this point is considerable. Parasites having only the same effective rate of increase as the host are of little practical interest, because the colony it is ordinarily possible to introduce is very small in proportion to the host population and requires an enormous time to increase to a point where it exerts any perceptible effect. It is, nevertheless, only with such species that repeated introductions can have any influence on the time required for control. The parasites, which reproduce more rapidly than their hosts, may be of great economic importance, but the effect of repeated introductions upon their increase is practically negligible. In short, we cannot help the useful species, while the species we can help are not useful. The only case in which repeated introductions are likely to be of any value is when a pest is present in several distinct colonies, separated by unpopulated areas or geographical barriers, over which the introduced parasites cannot pass.

Anything in the nature of encouragement of beneficial species, already present in an area, seems thus restricted to indigenous species. The only way of enhancing the value of such species is to produce artificially a considerable increase in their numbers in the field.

The possibility of producing such an increase is obviously limited in several ways.

Thus, a species, which can be used in the laboratory only during the period when it breeds in Nature, could not be profitably handled unless it were possible to bring about a decided increase, either in the number of generations or in the number of progeny introduced. The former possibility may at once be rejected, for it is seldom, if ever, possible to bring about an acceleration of developmental processes sufficient to allow of any marked increase in the number of generations normally produced. A marked increase in the number of progeny coming to maturity in Nature can probably be obtained in many cases by careful methods of rearing, especially with species having wasteful methods of reproduction, such as the Tachinids, which deposit eggs or larvae upon leaves or in the neighbourhood of the host; but even here the reproduction of a number sufficient to increase the population in the field to

a considerable degree, over a large area, would be extremely difficult and expensive. Furthermore, even if such a thing were possible, it would not necessarily be advisable. Thus, it would be of little value to liberate additional individuals of a parasite before a period or season in which adverse climatic or agricultural factors ordinarily cause the death of a large proportion of the population.

About the only beneficial insects we can "encourage" are, therefore, such species as can be bred easily and continually during periods when they are dormant in Nature, and their hosts either dormant or at least not present in a stage attacked by the parasite or predator reared. In order to carry on this breeding work a plentiful supply of hosts in the stage required must be available, and these hosts must be such as are easily and rapidly bred upon food material which is inexpensive and easy to procure. Thus, suppose we are dealing with a parasite attacking the larvae of the Oriental peach moth. Even if we could breed this insect during the winter, it would be quite unprofitable to do so if we had to supply it with larvae of the peach moth, reared for the purpose in peach shoots, grown in greenhouses or on peaches preserved in cold storage. The cost of such an experiment would, in all probability, far exceed the extra profit obtained by the work of the parasite reared.

Several successful, or at least promising, attempts in this direction, have been made. In California, the citrophilus mealybug is now controlled by liberating, in the beginning of the season, colonies of the ladybird, *Cryptolaemus*, which is bred throughout the winter in special insectaries. The food of the predators in this case, is the mealybug itself, but the cost of the operation is reduced by rearing the latter upon potato shoots. In England, Speyer[1] has for several years successfully reared *Encarsia formosa*, a Chalcid parasite of the greenhouse white-fly, on a large scale, for distribution to greenhouses in early spring, when fumigation sufficient to kill the white-fly is injurious to many plants. By an ingenious use of plants, unattractive to the parasite but attractive to the host, in combination with plants attractive to both, Speyer has bred *Encarsia* upon its normal host in enormous numbers; a most interesting and valuable achievement which has not yet received the attention it deserves. The latest development in this direction is the large scale breeding of the ubiquitous egg parasite, *Trichogramma*, for use against insects like the codling moth, the Oriental peach moth and other lepidopterous pests. In this case the egg parasite is bred throughout the winter on the eggs of one of the Lepidoptera breeding in stored

[1] Speyer, E. R., "The greenhouse white-fly," *Journ. Roy. Hort. Soc.* LIV, Pt 1, 1929.

products, such as *Ephestia* or *Sitotroga*, which can be reared with very little trouble on a colossal scale. By the use of ingenious devices, which have been described in a number of recent papers, a daily production of 1,000,000 *Trichogramma* has been reached, and there is no doubt that much larger numbers can be produced, since various phases of the work have now been standardised so that it can be carried on by a relatively unskilled assistant. The object in view in most cases is the liberation of large populations of the parasite in the field in the spring, at a time when its numbers are ordinarily small owing to the mortality during the cold or arid season. No really conclusive results have so far been obtained, but the prospects seem, on general grounds, to be better than in any project of this kind yet put forward, since it fulfils all the conditions we have seen to be necessary for success. It is true that there is in many Lepidoptera a high natural mortality in the early stages of larval development, so that the effect of increasing the percentage of parasitised eggs is much less than might be imagined; but this is to a great extent offset by the fact that the individuals destroyed are eliminated before they reach a destructive stage in their life history, so that the reduction in damage is immediate.

Time alone will show whether it is possible to increase the efficiency of any other types of parasites or predators in their native environments. It is of interest to note that one worker[1], who has experimented along this line, has found it possible to rear ladybirds on artificial food. If this method could be perfected and utilised practically, the large scale production of these useful insects might be possible.

At all events, it will be clear from what precedes that the "encouragement" of native parasites and predators, though obviously possible only under certain very definite conditions and necessarily restricted to a relatively small number of beneficial species, has nevertheless great possibilities, which have not yet been adequately examined.

(2) *Introduction of natural enemies absent from the area.*

(*a*) *With a known host in the area.*

(i) *Natural enemies not hitherto present.* The most important developments in biological control in modern times have been in the introduction of beneficial species into regions in which they have never before existed or are, at least, temporarily absent.

As is well known, the inception of this type of work is due to the arrival in various countries, of pests from other regions, which have

[1] Merritt Hawkes, O. A., *Proc. Zool. Soc.* pp. 475–90, London, 1920.

been introduced unaccompanied by the beneficial insects, which attack them in their native homes, and have increased rapidly to the point where they have become serious pests. In former times, when transport was very slow, and the practice of shipping food materials in cold storage was unknown, opportunities for the migration of injurious insects were not really very numerous. The modern development of rapid transport and cold storage allows of the exchange of food products between far distant points. The growth of world trade has permitted and fostered cosmopolitan tastes and requirements, which in turn further stimulate commerce between the various parts of the globe. The modern developments in transport and food preservation permit injurious insects to traverse, with relative ease, geographical barriers that were formerly impassable, under conditions which are exactly what they require. The rapid methods of locomotion bring them to new food supplies before the material in which they are being carried is exhausted, the cold storage facilities enable them to make the journey in a dormant state so as to issue in an active and healthy condition in their new home. As an example of the process it will be enough to say that, according to Herrick[1], of the 73 worst pests existing in North America over one-half have been introduced from foreign countries.

The attempt to control a pest of this type by introducing the beneficial organisms which prey upon it in its native home is based, of course, upon the supposition that the natural enemies play an important part as controlling agents in the country of origin, and that the increase and spread in the new home is due to their absence.

It is highly improbable that this simple hypothesis applies to all cases. As we have already said, control, whether partial or complete, is due simply to a departure from optimum conditions and may, consequently, be brought about in a great many ways. The complex of controlling factors differs in composition in different parts of the native home. The same parasites do not always exist at all points, and those that do often vary tremendously in importance in different zones. In some regions the effect of parasites is unimportant as compared with that of physical factors or cultural practices.

On the other hand, conditions usually vary considerably in different regions of the new home. The imported insect is not always uniformly destructive, being in some districts completely controlled and in all partially controlled by environmental factors. In other words, we have, both in the old and new homes of the pest, a vast number of complexes

[1] Herrick, G. W., *Manual of Injurious Insects*, New York, 1925.

of controlling factors. The controlling complex of the old home is not always A, and that of the new home always $(A-K)$; it may be, in the old home, A, B, C, D, etc., and in the new home B, D, F, G, as chance will have it. The difference between the controlling complexes of the old and new home is not a constant difference. Thus, although in the new home, considered as a whole, the imported pest increases and spreads, while in the old home it does not, it is difficult to conceive that this difference in its behaviour can have any simple and uniform cause.

Fortunately, however, the attempt to deal with the pest by the methods of biological control is in no way dependent on the solution of the difficult and delicate problem of the causes of its increase. Whether the parasites and predators, which attack the pest in its native home, are major or minor factors of control, and whether the increase in the new home is due chiefly to their absence or to a diminution in the intensity of some physical or cultural factor, is from the standpoint of the parasitologist, of relatively little importance. If parasites and predators attack the insect in its native home, he is quite justified in introducing them, since if they become acclimatised, they are certain to produce *some* diminution in the rate of increase and destructive powers of the pest.

A detailed study of the methods and practice of biological control cannot be undertaken in this paper. Certain authors have elaborated and published rules for the choice of beneficial species in work of this kind, but such rules are, in my opinion, of very little practical value. It is usually impossible to predict with any assurance the behaviour of an organism in a new environment, or to deduce, from observations on a parasite or predator in its native home, whether it will be a factor of minor or major importance in a new country.

It is sometimes asserted that if two species of parasites or predators tend to enter into conflict—as, for example, when one is capable of developing not only at the expense of the host but also as a hyperparasite of the other, only one of these and, preferably, the one which is an obligatory primary parasite, should be introduced. Pemberton and Willard[1] and, later, Willard and Bissell[2] have claimed that *Opius humilis*, the Braconid parasite of the Mediterranean fruit fly, introduced by Silvestri into the Hawaiian Islands in 1913, has been greatly reduced in effectiveness by two other fruit-fly parasites of the genus *Diachasma*, the larvae of which invariably destroy those of *Opius*, when both occur

[1] Pemberton, C. E. and Willard, H. F., *Journ. Agric. Res. Washington, D.C.* xiv, No. 13, 1918.

[2] Willard, H. F. and Bissell, T. H., *Ibid.* xxxiii, No. 1, 1926.

in the same host. According to the authors cited, *Opius* alone would have done more good than is now accomplished by this species and the two *Diachasma* working together, because of the loss through competition.

The data put forward by Pemberton, Willard and Bissell in support of their conclusions have, however, been subjected to a careful critical examination by H. S. Smith[1] in a recent paper dealing in a very comprehensive and thorough manner with the whole subject of multiple parasitism and its relation to biological control. In this paper Smith shows that on theoretical grounds, as well as on the data so far available, the policy of entomologists in introducing all available primary parasites of an injurious species is justified, and urges that it be continued.

The results of numerous dissections of a great variety of native insects made by the present writer during the course of a number of years' work on parasites, strongly support the conclusions of Smith. Parasites, like their hosts, are undoubtedly narrowly restricted in their increase and distribution by envionmental factors, but competition amongst species is certainly one of the least important of such factors. So far as the writer's experience goes, encounters between individuals of the same species are, on the whole, a great deal more frequent and a great deal more detrimental to its welfare than competition with other species. The difference in the habits and structure of different species tends naturally to produce a difference in distribution and thus diminishes the chance of conflict.

Therefore, in spite of the difficulties it entails, both in the collection of material and the study of results, the establishment of all the primary parasites of an introduced species it is possible to obtain seems advisable, although it seems best, on general grounds, to make the introductions successively, rather than simultaneously, bringing in new species only after the effect of those already established has been determined.

(ii) *Natural enemies hitherto present.* A special subdivision of the work in transferring beneficial insects from one area to another concerns cases where the absence is temporary, the species in question having inhabited the area in the past. A good example of this type of work was the distribution to farmers by S. J. Hunter of Kansas of *Lysiphlebus tritici*, the Braconid parasite of *Toxoptera graminum* or "Green Bug," in 1907 and 1908[2]. According to this author, the green bug becomes active in spring at a lower temperature than its parasite and is carried

[1] Smith, H. S., "Multiple parasitism: its relation to the biological control of insect pests," *Bull. Ent. Res.* xx, Pt 2, pp. 141–9, August 1929.

[2] "The Green Bug and its natural enemies," *Bull. Univ. Kansas*, ix, No. 2, 1909.

by the wind to regions where the latter is absent and to which it does not follow the host until the latter has already become so abundant as to cause serious damage. He claimed that, by the artificial distribution of the parasite, this delay could be eliminated and the host held in check. However, the report published by Hunter, though containing much interesting data, provides no satisfactory evidence in support of the statement that the green bug is able by migration to escape from its parasites. According to Howard and Fiske (*l.c.*), elaborate experiments along the same lines were made by Webster but no definite results were obtained, because the parasite, which develops in other species of plant lice, occurs naturally throughout the whole range of the green bug.

It is possible that the importation of parasites normally present in an area may under certain exceptional conditions be useful or even necessary. There are, however, two serious difficulties connected with this type of work. In the first place, it is extremely difficult to be certain that the species introduced is really absent from the area, especially if the examination is made at a time during which it is inactive. Anyone who has attempted to find hibernating insects knows that in many cases the most careful search fails to reveal them in areas in which they are known to be present and appear in large numbers as soon as conditions become favourable. In the second place, when species are temporarily rare in an area or absent from it, the reason usually is that conditions are temporarily adverse to it, and tend to maintain a low population level. Under such circumstances attempts to reintroduce the species or artificially increase its population are not likely to give any good results.

The only cases in which reintroduction is justified are those in which some catastrophic factor of a very unusual type—such as an abnormal drought or an exceptionally hard winter—has practically or absolutely exterminated a beneficial insect in a region so situated that repopulation from adjacent areas in which it still occurs, is impossible, or can occur only very slowly owing to the existence of barriers of some kind. It is obvious that a very careful preliminary study is desirable before any great expenditure is made on projects of this kind.

(*b*) *With no known host in the area.*

When the economic entomologist has to deal with a very injurious pest, which is indigenous and only partially controlled by its native parasites, he may attempt to rectify matters by introducing entomophagous insects attacking pests belonging to allied species which exist in other countries.

If the beneficial insects introduced are extremely specific in their host relations they will, of course, be unable to establish themselves on the allied host. However, a large number, and perhaps the great majority of parasitic insects, are capable of developing in hosts belonging to many species. The fact that imported insects, as for example the Oriental peach moth, are sometimes fairly heavily attacked by native parasites, shows that the host-parasite relationship is often flexible enough to bring together species never associated in the past. It has sometimes been stated that polyphagous parasites are, in general, of little importance in natural control; but there does not seem to be any solid ground for this assertion. The importance of *Compsilura concinnata* and *Apanteles glomeratus* as controlling agents and the evident unimportance of such specific parasites as the dipterous enemies of myriapods and woodlice, proves that the degree of specificity is not always a reliable index to the value of a parasite or predator as a controlling agent. In the opinion of the writer, the transfer of beneficial insects to regions in which possible hosts exist is a quite promising field for investigation.

VI. The results of experiments in biological control.

In spite of the numerous experiments which are being made in biological control, we seldom find in articles treating of this subject any clear cut statement as to the results that may be expected. It therefore seems worth while to devote a little space to the analysis of the action of entomophagous insects from the economic standpoint.

The starting point of experiments on biological control is practically always the same and consists in the liberation, in an area containing vast numbers of host insects, of a small number of individuals of a beneficial species. It is clear, and is generally realised, that no results of great economic importance can follow until the beneficial species has "overtaken" the host or, in other words, until the difference between the populations of the host and parasite has very greatly decreased. To make clear exactly what may be expected under various conditions, a more detailed survey of the subject is necessary.

At the moment when the beneficial insect is liberated the host insect may be:

(1) Still increasing in numbers; or

(2) It may have increased to the limit permitted by environmental conditions so that its population remains stable, or, at most, oscillates around a certain average figure.

If the host population is increasing—as in the initial period of an invasion—then, as has been shown in previous papers, the parasite will never overtake the host, unless the effective reproductive rate of the former is equal to or greater than that of the latter. If the reproductive rates of the host and parasite are equal, the parasite will eventually overtake that host, but the process will be very slow, the number of generations required for control being found by dividing the number of reproducing hosts by the number of reproducing parasites—other things being equal; so that a colony of 1000 parasites will require 1000 generations to overtake a population of 1,000,000 hosts if the effective rates of increase of the two are equal. If the reproductive rate of the parasite is greater than that of the host, the time required for control will be considerably lessened. A slight superiority in reproductive rate, other things being equal, confers a great advantage. Thus a population of 1000 parasites, reproducing one and a half times as fast as the host, will overtake a pest having a population of 1,000,000 in only 14 generations.

Under the conditions postulated, and supposing that all of the individuals of the host are accessible to attack by the parasite introduced, the moment when the parasite overtakes the host will, theoretically, be marked by the total extermination of the latter, followed by that of the parasite, or in a practical case, by a great reduction of the host population, after which the parasite population would also be decimated owing to the rarity of hosts, the condition thus produced forming the point of departure of a new cycle of development.

However, in spite of the fact that the host is ultimately exterminated by the parasite, and that the percentage of hosts destroyed increases from generation to generation, no reduction whatever in damage will be produced until the generation in which extermination occurs. On the contrary the host will steadily increase in numbers and consequently, in destructiveness, and though its rate of increase from generation to generation will be gradually diminished, the general situation, considered from an economic standpoint, will steadily become not better, but worse and worse until the climax is reached.

It is true that certain types of entomophagous insects, such as the egg parasites and some of the predators, destroy their hosts before they have reached the destructive stage, whereas others and indeed the great majority, do not produce death until after the feeding period. Those of the former type will of course slow down an increase in destructiveness slightly more than those of the latter type. A pupal parasite, or one issuing from the full-grown larva simply prevents its host from repro-

ducing; an egg parasite not only prevents it from reproducing but also prevents it from doing any damage. Nevertheless, in the particular case we are considering, the presence and increase of the parasite will not prevent the increase in numbers and destructive power of the host. The increase in damage will indeed be less than it would be if the parasite were not present, but it will be distinctly greater than it has been in the past periods when the parasite was not present.

If the host population has reached its maximum and become stable and the introduced parasite or predator is capable of increasing in its new home, then immediate benefit may be expected, provided that the reproductive power of the host has not been checked because its population is too large for the area (in which case the loss from parasitism might, at least for some time, be counterbalanced by the increase in reproduction due to the fact that more spots are available for the offspring) and provided also that the individuals destroyed will not naturally succumb, even in the absence of the parasite, through the action of other controlling factors (in which case the action of the parasites would have no effect until they became abundant enough to kill individuals which would otherwise survive). If the host is killed in the predestructive stage, the diminution in the damage produced will naturally be more rapid than when it is killed at the end of the destructive phase in its life history. But in any event, under favourable circumstances, the action of the introduced parasites or predators on hosts with stable populations should produce an immediate if gradual reduction in economic damage.

It is, however, not to be expected that the reduction in damage effected by any single species will occur uniformly over the whole of an infested area. Neither entomophagous nor phytophagous insects are capable of existing in every point in which food material is available and different species utilising the same food frequently differ greatly in their distribution. Therefore, even though the introduction of a parasite produces beneficial results at one point, it may be of little value in another, so that not only may it be necessary to search for and introduce other entomophagous enemies of the pest, but it may also be essential to supplement their efforts with the methods of mechanical, chemical or agricultural control. The methods of biological control have in many cases proved their value, but they are not infallible; and there is no reason to suppose that they will ever supplant all other methods of combating insect pests.

VII. Summary.

1. In view of the rapid development of practical experiments in biological control, a general survey of the subject seems desirable. The foregoing paper designed to meet this need contains a discussion of the nature of biological control, the character and practical value of biotic controlling factors of various types, the pests to which the method can be applied, the situations in which it can be utilised and the results that may be expected from it.

2. By *control* we mean a check on the increase of an organism brought about by any cause whatever. By *biological control* we mean the reduction of the rate of multiplication of an organism effected through the agency of other organisms as distinct from non-living factors.

3. There is no necessary connection between control as a biological phenomenon and control in the economic sense.

4. The complex of factors responsible for the control of an organism differs in composition both qualitatively and quantitatively in the various districts inhabited by it; the measures necessary to re-establish control, in the case of an outbreak, will also vary in different times and places. It would, therefore, be unwise to rely exclusively upon the methods of biological control for the solution of entomological problems.

5. The main factors of the biological control of insect pests are: (1) Pathogenic organisms; (2) Invertebrate parasites and predators; (3) Vertebrate predators.

6. Pathogenic organisms are sometimes very effective but practically impossible to manipulate successfully. Among the parasitic and predaceous Invertebrates, the entomophagous insects are by far the most important and the most useful. Vertebrate predators apparently play only a minor part in the control of insect pests, and there is little indication that they can be utilised in practice.

7. The control of insect pests by the use of entomophagous insects seems, on the whole, much easier to bring about than the control of plant pests by phytophagous insects, in the first place because the insect is much more susceptible to injury than the plant, and in the second place because the entomophagous insect, being of about the same size as its host, is capable of inflicting greater injury than the phytophagous insect can inflict upon the host.

8. The attempt to control plant pests by phytophagous insects is more dangerous than the attempt to control insect pests by entomophagous insects, because if the phytophagous insect changes its host

after introduction it may attack a useful plant, whereas the entomophagous insect, even if it does attack hosts other than that against which it was introduced, is not likely to attack any beneficial insect.

9. The object of work in biological control may be either:

(i) the intensification in the action of natural enemies already present in the area; or

(ii) the introduction of natural enemies (*a*) temporarily absent from the area; or (*b*) never before present in it.

In the latter case the species introduced may be either (i) species of which a known host already exists in the area; or (ii) species of which no known host exists in the area.

In general, the most promising type of work concerns introduced species from other areas, though valuable or, at least, promising results have been obtained in the work with certain established species.

10. The results which may be expected from experiments in biological control depend primarily (other things being equal) on whether the host is increasing in numbers or not. If it is increasing, the increase of the parasite at its expense will not cause a reduction in damage or a decrease in numbers, which will not occur until the parasite has overtaken the host. If the host population has become stabilised, then the increase of the beneficial species at its expense will give immediate relief, though this may be slight at first. The reduction of damage will continue until the rarefaction of the host population begins to cause a diminution in the effective reproductive rate of the parasite, after which the host population will again increase for a time.

11. Generally speaking, no one species of parasite or predator is likely to bring the host under control over the whole of the infested area. To produce this result, the introduction of additional species will usually be necessary, while in many cases, their efforts must be aided by the methods of agricultural, chemical or mechanical control.

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ON THE BIOLOGY OF THE GALL-MIDGES (CECIDOMYIDAE) ATTACKING MEADOW FOXTAIL GRASS (*ALOPECURUS PRATENSIS*), INCLUDING THE DESCRIPTION OF ONE NEW SPECIES

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CONTENTS.

1. Introduction

Meadow foxtail grass (*Alopecurus pratensis*) was formerly used to a considerable extent in seed mixtures for pasture and meadow land. Latterly, however, its use has greatly diminished and timothy or meadow cat's tail grass (*Phleum pratense*) has taken its place. One important reason for this change was that seed merchants had difficulty in supplying good meadow foxtail seed. This failure to be able to obtain good seed was due to the very large percentage of the florets that either appeared to the seed testers to contain grubs or else appeared to be completely empty, either of seed or anything else[1]. The grubs that are present are larvae of the Dipterous family Cecidomyidae and may be either one or

[1] Cf. Cockayne (1916): "The lowness of germination is generally due to the fact that all lines, although well cleaned from the point of view of extraneous seeds, contain a large percentage of empty husks and immature kernels."

both of two species. One reason for the enormous prevalence of empty florets or blind seed cases is the fact, as will be shown later, that a third species of gall-midge larvae inhabits the florets only for about 3 to 5 weeks during the summer, and then migrates to the soil. Hence all that the seed testing station would receive would be empty florets and immature kernels.

At present most of the seed that is used in this country comes from Scandinavia and Finland where they also have the midges present, while Russia and Holland also export a little.

This study was commenced in 1926 when Prof. S. P. Mercer of the Seed Testing Division, Queen's University, Belfast, sent some infested seed to have the larvae identified. It was therefore started while the writer was at the S.E. Agricultural College, Wye, under the late Mr F. V. Theobald and continued at Rothamsted under Dr A. D. Imms. The writer wishes to thank these three persons for their help and advice and to all who have been good enough to send samples of the grass, without whose assistance this study could not have been done.

2. Methods.

Letters were sent to persons living in various parts of Great Britain requesting them to send about 100 heads of the grass. As soon as these samples were received, ten heads were kept apart in order to estimate the number of florets in the sample of foxtail. The remaining heads were placed on soil in flower pots and covered with a lamp glass, which in turn was covered on top by muslin sewn to an iron ring. These pots were placed in the insectary and watered at intervals. The emergence of midges and parasites was observed for 12 months or more. In this way the distribution of the midges and parasites was noted, as well as the degree of relative infestation.

In order to ascertain the amount of success in rearing midges from such material, similar pots containing 1000 larvae of known species were set up. This also provided a means of discovering what species of parasite attacked any particular species of midge.

For the experiments concerned with oviposition and duration of the egg stage, plants were put in muslin covered pots before the midges started emerging, thus making certain they were free from any attack, and midges of known species put in at intervals.

Emphasis should be laid on the large number of field observations made as a control to those conducted in the insectary. These field observations are considered most essential.

3. SPECIES OF MIDGES INVOLVED AND THEIR DISTRIBUTION.

Two species of midges have been known for a number of years[1] to prevent the seeding of meadow foxtail grass, namely, *Dasyneura alopecuri* (Reuter) and *Stenodiplosis geniculati* Reuter, both first described from Finland in 1895. A third species, *Contarinia merceri* n.sp., is here described for the first time. A possible reason why this midge has been previously overlooked is the fact that the larvae do not overwinter in the seeds and so do not appear in seed samples. It appears to be present in far greater numbers than the two other species and also to be responsible for the so-called "blindness" of such large quantities of the grass.

In addition, at least one species of *Lestodiplosis*, possibly two, is to be found occasionally in the flower heads.

Distribution.

These midges seem to flourish wherever meadow foxtail grass grows. They are to be found throughout the British Isles and are found in Finland, Denmark, and one species at least in New Zealand. A detailed survey of their distribution in England has been made, and from the accompanying table it will be seen that in every sample received (except in one from Surrey) at least one species of midge larva occurred. It will also be seen that the most prevalent species is *C. merceri*, while *D. alopecuri* is next in abundance.

Samples have also been received from Denmark, Finland, Scotland, Ireland, the Isle of Man, the Orkneys and Majorca. In the two last-named samples no midge larvae were found, yet there was a very large number of empty florets indicating the probability that *C. merceri* had been present. The results of examining the other samples is to be found under the separate species. The absence of any of the midges in any one sample may be due to collecting the sample at a date when all the midges are not present in the seeds; thus, it must not be inferred that *C. merceri* is not present in Langwathby, Cumberland, it is more likely that the larvae of this species had "jumped" to the ground by July 21st, 1928.

[1] The first known record of foxtail grass seed being damaged by midge larvae is as follows: Mr H. Gibbs, at a meeting of the Royal Agricultural Society of England held on May 5th, 1841, stated "all the Foxtail-grasses were more or less subject to be infested with a small orange-coloured larva which preyed upon the germ, and destroyed the vitality of the seed to such an extent that in most cases not more than one seed in a dozen ever vegetated on sowing" (*Gardeners' Chronicle*, I, 311, 1841).

Table I.

Survey of distribution of midges in England.

County	Locality, date and collector's initials	*D. alopecuri*	*C. merceri*	*S. geniculati*
Bedfordshire	Henlow 6. vii. 29, A.D.I.	×	×	.
	Biggleswade 6. vii. 29, A.D.I.	×	×	.
	Bedford 7. vii. 29, C.W.H.	.	×	.
Berkshire	Reading 8. vii. 29, F.O.M.	×	×	×
Buckinghamshire	—	.	.	.
Cambridgeshire	Cambridge 2. vii. 29, R.H.B.	×	×	×
Cheshire	—	.	.	.
Cornwall	Launceston 7. vii. 29, E.G.T.R.	×	×	.
Cumberland	Langwathby S.O. 21. vii. 28, H.B.	×	.	×
Derbyshire	11. vii. 29, A.R.	×	×	.
	Two Dales 5. viii. 28, H.F.B.	.	×	.
Devonshire	Newton Abbot 20. vi. 27, W.E.H.H.	.	×	×
	Newton Abbot 19. vi. 28, C.A.C.	.	.	×
Dorsetshire	Corfe Castle 26. vi. 29, L.B.H.	×	×	×
Durham	Durham 14. vii. 28, B.M.G.	×	×	×
Ely, Isle of	—	.	.	.
Essex	Churchyard, Mucking 17. vii. 28, C.R.N.B.	×	×	.
	Marshes, Stanford le Hope 18. vii. 28, C.R.N.B.	×	×	.
Gloucestershire	Willersey, 19. vii. 28, J.M.	×	×	×
	Buckland 19. vii. 28, J.M.	×	×	×
Hampshire	Basingstoke 1925, M. of A.	×	.	.
Herefordshire	—	.	.	.
Hertfordshire	Rothamsted 23. vi. 27, H.F.B.	×	×	×
Huntingdonshire	—	.	.	.
Kent	Wye 14. vi. 27, S.T.P.	×	×	×
	Wye 15. viii. 27, S.T.P.	×	.	.
Lancashire	—	.	.	.
Leicestershire	11. vii. 27, A.R.	×	×	×
Lincolnshire	19. vii. 27, A.R.	×	×	×

Table I (*continued*).

County	Locality, date and collector's initials	*D. alopecuri*	*C. merceri*	*S. geniculati*
Middlesex	Pinner 16. vii. 28, R.P.	×	×	·
Monmouthshire	—	·	·	·
Norfolk	Thetford 1. vii. 27, W.M.D.	×	×	·
Northamptonshire	—	·	·	·
Northumberland	Newcastle 7. vii. 27, R.A.H.G.	×	×	×
Nottinghamshire	19. vii. 27, A.R.	×	×	×
Oxfordshire	Oxford 2. vii. 29, A.H.H.	×	×	×
Rutlandshire	Oakham 19. vii. 27, A.R.	×	×	×
Shropshire	Newport 12. vii. 27, S.G.J.	×	×	×
Somersetshire	Williton 1. vii. 29, A.R.	×	×	×
	Mucholney nr Longport 2. vii. 29, C.L.W.	×	×	×
	Sidmouth 18. v. 28, R.B.	×	·	·
Staffordshire	—	·	·	·
Suffolk	Wickhambrook 1. viii. 28, H.F.B.	×	×	·
Surrey	Wisley 13. vii. 28, G.F.W.	×	·	·
	Roadside, Ripley 16. vii. 28, G.F.W.	·	·	·
	Riverbank, Wisley 2. vii. 29, G.F.W.	×	×	×
	In shade, Ripley 3. vii. 29, G.F.W.	×	·	·
	Wimbledon Common 7. vii. 29, E.L.	×	×	·
Sussex	Pasture, nr Willingdon and Hampden Park 27. vii. 29, R.A.	·	×	×
	Roadside, Hampden Park, nr Eastbourne 26. vii. 28, R.A.	·	×	×
Warwickshire	—	·	·	·
Westmoreland	—	·	·	·
Wight, Isle of	Freshwater Bay 25. vi. 29, J.G.	×	×	×
Wiltshire	Marlborough 22. vii. 28, L.G.P.	×	×	·
Worcestershire	—	·	·	·
Yorkshire	Leeds 11. vii. 27, T.H.T.	×	×	×
	Scarborough 24. vii. 28, B.H.B.	×	×	×

4. Keys for identification of larvae, pupae and adults.

The larvae of the midges may be separated into those species which possess an anchor process and those that do not. All the species are distinguishable by their colour. The following key may be used for their separation.

A. Larvae with anchor process.

- B. Larvae cadmium orange (Ridgway, 1912), anchor process with sharp points . . . . **Dasyneura alopecuri** (Reuter)
- BB. Larvae deep chrome to cadmium yellow (Ridgway), anchor process with rounded points . . . . **Contarinia merceri** n.sp.

AA. Larvae without anchor process.

- B. Larvae light ochreous salmon to light ochreous buff to warm buff (Ridgway) . . . . **Stenodiplosis geniculati** Reuter
- BB. Larvae blood red . . . . **Lestodiplosis** sp.

The empty pupal cases may be separated as follows:

A. To be found in the flower heads.

- B. Wing cases, head and thorax very dark, abdomen hyaline **Stenodiplosis geniculati** Reuter
- BB. Pupa entirely hyaline . . . . **Dasyneura alopecuri** (Reuter)

AA. Not to be found in the flower heads, in the soil.

- B. Wing cases, head and thorax slightly darker than abdomen **Contarinia merceri** n.sp.
- BB. Pupa entirely hyaline . . . . **Lestodiplosis** sp.

The adult midges found ovipositing on meadow foxtail grass heads may be separated as follows:

A. Ovipositor pocket-shaped, claws toothed **Dasyneura alopecuri** (Reuter)

AA. Ovipositor lamelliform, wings speckled, claws simple . **Lestodiplosis** sp.

AAA. Ovipositor needle-shaped, claws simple.

- B. Palpi 4-segmented, long . . . . . . **Contarinia merceri** n.sp.
- BB. Palpi 3-segmented, short . . **Stenodiplosis geniculati** Reuter

5. *Dasyneura alopecuri* (Reuter).

(*a*) *Synonymy and history.*

1895 Reuter, E., *Act. Soc. Fauna and Flora Fennica*, XI, 6–9 (*Oligotrophus*).
1927 Barnes, H. F., *E.M.M.* LXIII, 214–15 (*Dasyneura agropyronis*).

Curtis (1860) refers to a midge in meadow and slender foxtail which probably was this species, while Miss Ormerod (1885) figures the anchor process.

This species was described by Reuter in 1895 as *Oligotrophus alopecuri*; in his description he states that the claws were simple and the body colour of the midge honey yellow. Specimens of males and females, labelled "*O. alopecuri* Reuter, Denmark, Rostrup" were very kindly sent by Dr R. Frey from the Museum Zoologicum Universitatis, Helsingfors, Finland, for critical examination. The specimens were very much cleared and macerated. There was a slight indication of a tooth on the claws. It may be presumed that these were some of the specimens sent by Rostrup to Reuter (Rostrup, 133 Beretning fra Statens, etc., 1919).

Another set of specimens was received from Mag. P. Bovien, Lyngby, labelled "*O. alopecuri* ? S. Rostrup, 1912–13, Denmark." In some of these the claws are distinctly toothed while in others the tooth is almost invisible.

Kieffer (1913) doubtfully refers *alopecuri* Reuter to the genus *Oligotrophus*. However, all the specimens reared by the writer have toothed claws and are typical *Dasyneura*. Therefore it is now proposed to transfer *alopecuri* Reuter to the genus *Dasyneura*.

The writer in 1927 described *Dasyneura agropyronis*, a midge found swarming in large numbers on *Agropyronis repens* in July 1924 by Dr G. D. Morison. At the same time large numbers of parasites were observed. This species, both from the specimens of midges and the presence of the parasites, is undoubtedly *Dasyneura alopecuri* (Reuter), and is therefore now sunk as a synonym.

(*b*) *Description.*

Reuter's original description of this species is adequate, except that it should be pointed out that the claws are usually distinctly toothed, the body colour red and covered usually with bands of scales. The midge is a typical *Dasyneura* with black scales on the red body. The number of antennal segments varies from $2 + 11$ to $2 + 16$ in the males and from $2 + 10$ to $2 + 15$ in the females. The males are darker than the females, whose abdomens are bright red when full of eggs and duller when oviposition is over. There is, in addition, much variation in size of both sexes.

(*c*) *Distribution.*

This species is found throughout the counties of England (see Table I), Ormerod (1885) reporting it from Cheshire. It has also been found in samples from Glamorganshire, Cardiganshire and Caernarvonshire in Wales; near Aberdeen and Newburgh in Scotland; the Isle of

Man; in counties Antrim, Armagh, Tyrone, Louth, Dublin, Wicklow and Wexford in Ireland.

It also occurs in Finland (Reuter, 1895) and Denmark (Ferdinandsen and Rostrup, 1920).

It is reported in New Zealand (Miller, 1918) as a serious pest.

(*d*) *Bionomics.*

Emergence. The adult midges as a general rule emerge before noon (sun time). The majority emerge between 8 and 11 o'clock in the morning, the males appearing slightly before the females, and nearly all the males have emerged by 11. The females continue emerging until about 4 in the afternoon, although the vast majority have emerged by noon. Then at about 7 in the evening there is another emergence of males; this is the chief difference between the emergence of the males and females. These statements find support in the following figures[1] taken from emergences in 1928. They deal with the emergence of 4082 males and 6034 females.

	Before noon				After noon			
	Males		Females		Males		Females	
	Actual No.	%	Actual No.	%	Actual No.	%	Actual No.	%
Eight English samples	2119	$88 \pm 1{\cdot}68$	3757	$97 \pm 0{\cdot}65$	279	12	122	3
Five Irish samples	1445	$86 \pm 2{\cdot}56$	2079	$96 \pm 0{\cdot}68$	239	14	76	4

Light probably is a factor controlling emergence, as is indicated by the following experiment. A sample of foxtail seed infested with this midge's larvae was kept in the dark until noon (sun time) each day from about 8 the previous evening, thus giving additional darkness. This procedure was started at the time the midges started emerging. The

	Before noon				After noon			
	Males		Females		Males		Females	
	Actual No.	%	Actual No.	%	Actual No.	%	Actual No.	%
One sample	591	74	546	70	210	26	234	30

[1] It has been pointed out that by counting eight samples together erroneous percentage figures are obtained. The following figures are the male percentages before and after noon in the eight samples from England: 80 and 20, 86 and 14, 85 and 15, 95 and 5, 90 and 10, 86 and 14, 93 and 7, 88 and 12. For the females 96 and 4, 94 and 6, 97 and 3, 97 and 3, 97 and 3, 100 and 0, 95 and 5, 98 and 2.

result was that the percentage of midges emerging after noon was raised as the figures show; 801 males and 780 females emerged.

Other factors, such as temperature, undoubtedly affect emergence, and a subsequent paper will deal with the factors affecting the emergence of midges.

Emergence in the field varies in date with the locality; in the more southern and western districts the midges emerge earlier in the season (*e.g.* Kent 1927, May 30th; Co. Louth 1928, May 5th; Somerset 1928, May 18th; Hertfordshire 1928, May 3rd) than those in the more northern districts (*e.g.* Aberdeen 1924, July). This difference is completely nullified by keeping all the samples from different localities in one locality from the time the larvae are full grown until the midges emerge. In Hertfordshire in 1928 the earliest emergence noted occurred on April 27th and the latest on July 20th, while the maximum number of emergences occurred on June 11th. In 1929 the crest was about June 6th–7th. The date of emergence varies to some extent with the "earlyness" or "lateness" of the season.

As has been stated above, the males are usually waiting for the females to emerge. As soon as this occurs the females run up a blade of grass, and by the time they have climbed a few inches above the ground the wings are full-sized. They extend the ovipositor at full length once or twice, perhaps to make certain it is in working order or to aid in drying. Then they rest quietly on the blades of grass with the ovipositor inserted.

Mating. As soon as a male happens to come within a foot or two of the females, the latter immediately protrude their ovipositors to full length. This realisation of the presence of the males by the females is at once striking and accurate. It has been observed, experimentally, that the females can "scent" or "feel" the presence of males at least a yard away, and that they differentiate between males of their own species and other species of the same genus.

The males also appreciate the presence of the females in the same way, with the addition that they flutter and run wildly up and down the grass stems, tumbling madly over all obstacles in their efforts to find the virgins. Substitute an inseminated female and all excitement vanishes.

Very soon the male finds the female and insemination takes place without ceremony. In one case a male was placed in a cage about 2 ft. high with a diameter of about a foot, in which there were already three virgin females. The male appeared literally mad, fluttering, scrambling, flying in all directions. It appeared utterly confused by the presence of three females, although this is a state that would often occur naturally.

Within 8 minutes it was inseminating a female. Coition actually lasts about 30 seconds. After completion the male will pause for about 5 minutes and then hunt for another female. It has been observed that one male will mate with at least two females.

Sex ratio. Out of 6144 emergences in 1928 from English, not including Rothamsted, samples, there were 2675 males and 3469 females, giving a ratio of 44 males to 56 females. Again, out of 4317 emergences in 1928 from Irish samples, there were 1826 males and 2491 females, giving a ratio of 42 males to 58 females.

Oviposition. After mating, the females rest a short time and then proceed to the flower heads and start egg laying. They will lay their eggs on heads that show the anthers, or show stigmas, or show neither. The long ovipositor is thrust between the glumes, and the eggs laid singly on the inside of the glume enclosing the ovary. In captivity sometimes the eggs are laid within the outer sheath, and sometimes on the outside of the florets close down to the central stalk; in these latter positions the females will occasionally lay several eggs, up to seven being observed. There does not seem to be any marked crest of oviposition as in the case with *C. merceri* for the females lay soon after emergence and go on all day.

Duration of egg stage. The egg stage under favourable conditions lasts from 7–8 days, but in cold spells may be prolonged to 18 days.

Description of egg. The eggs are pale red and elongate oval in shape. If extracted from an inseminated female they are about 0·42 by 0·10 mm. in size, but 2 hours after being laid they measure 0·34 by 0·05 mm. After 24 hours they are 0·36 by 0·07 mm.

Larva. On emergence the larvae are very pale biscuit in colour, but later attain a cadmium orange (Ridgway) colour. In other terms, they may be described as brick-red to deep orange. In shape the full-grown larvae are almost oval, being greatly constricted in length and in sharp contrast to the more active species. The larva remains in the same floret, and never moves away once it has taken up its position on the seed. As a matter of observation of 1382 instances of florets infected in the field, in only three florets was more than one larva (in each case two) found in one seed and, in those exceptional cases, the second larva was very small and dying. The larvae feed up quickly and attain their maximum size in from 6 to 8 weeks. Then they enter a quiescent stage until about 8 days before the adult midges emerge. This means that full grown larvae are to be found in the floret, wherever it happens to fall, from July until the following May.

Pupa. The pupal stage lasts about 8 days, and pupation takes place in the seed case. When the midge is ready to emerge, the pupa wriggles up the seed case between the glumes until it is protruding about as far as the thorax. It bends sharply over backwards and forwards at the junction of the thorax and abdomen until a split occurs down the middle of the thorax. No midge pupa has been observed to bore through the glumes, only between them. The small holes to be seen in infested seed cases are the emergence holes of the parasite, *Prosactogaster attenuata* Hal.

Broods. The species is single brooded. The life-cycle may be briefly summarised as follows[1]: egg, 8–10 days in May; larva growing in size 6–8 weeks, May to July; developing larva, July to May; pupa, 8 days in May; adult up to 5 days. In two cases out of 13,193 midges, single males have been known to emerge the same year as the egg was laid. One emerged from a Rutland sample on August 24th and the other from a Kent sample on August 9th. This indicates that there is a possibility that in very favourable seasons occasionally there may be a second brood. It is not suspected that this is a regular occurrence in this country.

Variation in duration of life-cycle. As will be seen from the foregoing remarks, the usual duration is about 1 year but may be shortened to about 4 months. On the other hand, it may also be lengthened very considerably. From a sample of foxtail seed collected in co. Tyrone on August 16th, 1926, and which was kept moist in a glass top tin box, midges emerged from June 7th, 1927, until July 4th, 1927. The sample was then kept dry with no further attention, in order to see whether any midges would emerge after the second winter. This is highly important in control work, as Rostrup (1919) suggests keeping the seed over for a year as the midges emerge during the first year and apparently the germinating power of the seed does not diminish. However, between January 26th, 1928, and February 14th, 1928, three more male and three more female *D. alopecuri* midges emerged. Thus the duration of the life-cycle was increased to about 22 months. In this case as well, while parasites emerged throughout the summer of 1927, two also emerged during February 1928 and one in June 1928. This prolongation probably was due to the dryness, cf. Hessian fly. It, however, has never been repeated.

Variation in adult. In one instance an albino female was reared from a sample of foxtail from Finland. In this case the female (Cecid. 1183) was normal except for a complete lack of the red pigment in the abdomen, which was white with the usual black scales.

[1] Months correct for Harpenden, later for localities farther north.

The number of antennal segments varies from 2 + 11 to 2 + 16 in the males, and from 2 + 10 to 2 + 15 in the females. One freak female (Cecid. 1213), in which there were 2 + 4 antennal segments, was bred from a Newcastle sample. Variation and abnormality of antennal segments in midges will be the subject of a subsequent paper.

One specimen with normal male antennae, yet with ovipositor, has been reared under normal conditions in 1929 from an Aberdeen sample. This is the only case that has been noticed out of about 55,000 normal reared specimens. This phenomenon, although rare, has been known to occur in two other cases in Cecidomyidae, *i.e.* in *Mayetiola phalaris* Barnes (male antennae + ovipositor, Cecid. 225) and *Rhabdophaga heterobia* H.Lw. (female antennae + male genitalia, Cecid. 1206).

6. *Stenodiplosis geniculati* Reuter.

(*a*) *Synonymy and history.*

1895 **Reuter, E., *Act. Soc. Fauna and Flora Fennica,* xi, 10–14.**

This species was first described from Finland by Reuter in 1895. Dr Felt in December 1926 gave the writer a slide (Cecid. 434) of *Stenodiplosis geniculati* ♂, ♀ collected on *Panicum* in 1918–19 at Los Banos, Luzon, Philippine Islands. This was a misidentification and the midges are certainly not *S. geniculati* Reuter.

(*b*) *Description.*

Reuter's original description and figures serve for the second brood of this midge which overwinters. The summer brood, however, is much lighter in colour, the abdomens of the females being bright red. This species is a typical *Stenodiplosis*, no variation being known in the antennae and not much in size.

(*c*) *Distribution.*

S. geniculati occurs throughout England (see Table I), Bagnall and Harrison (1922) recording it from Durham and Yorkshire. It also has been found in samples from the Isle of Man, Wales (Glamorganshire), and Ireland (counties Antrim, Armagh and Tyrone). It is also to be found in Finland (Reuter, 1895).

(*d*) *Bionomics.*

Emergence. This species emerges earlier in the day than the last species, at least in the summer brood. For instance on one day (June 29th, 1927) out of 123 males that emerged, 72 had emerged by 5.45 a.m.

(sun time), 17 by 7 a.m., 27 by 9 a.m., and 7 by noon; similarly, out of 126 females that emerged on the same day, 30 had emerged by 5.45 a.m., 25 by 7 a.m., 20 by 9 a.m., 41 by noon, 9 by 5 p.m., and 1 between 5 and 6.30 p.m. Here again the males emerge slightly before the females, and in both sexes the majority emerge by noon. However, there is not the evening emergence that was observed to take place in *D. alopecuri*. The following figures support the above statements. The emergences recorded occurred in a sample sent from Devon in 1927.

By 9 a.m.				By 1.30 p.m.				By 9 p.m.			
Male		Female		Male		Female		Male		Female	
Actual No.	%	Actual No.	%	Actual No.	%	Actual No.	%	Actual No.	%	Actual No.	%
880	81	764	58	173	16	452	32	32	3	128	10

Sex ratio. Out of 4303 emergences in 1927 of the summer brood from English samples, there were 1999 males and 2304 females, giving a ratio of 46 males to 54 females. It is very difficult to obtain accurate figures for any sex ratio, but occasionally the figures are so abnormal that a sex linked lethal is suspected. For example, in a Lincolnshire sample, out of 148 emergences, 104 or 70 per cent. were females, thus suggesting that half the males never reached maturity. This suggestion is merely tentative. How easy it is to jump to a conclusion when dealing with emergences from random samples of seed heads is shown by the fact that in one sample from Derbyshire 69 females and only 7 males of *D. alopecuri* emerged during the first 18 days that midges were emerging, and yet at the end of emergence the ratio was normal. More concerning sex ratio will be included in the paper on emergence of midges that is being prepared.

Larvae. The larvae of this species are light ochreous to light ochreous buff to warm buff (Ridgway). In other terms, they are pale biscuit or honey yellow. They may be distinguished from the larvae of other species to be found in meadow foxtail heads by possessing no anchor process, a character possessed only by *Lestodiplosis* sp. among species whose host plant is this grass. The larvae in shape are slightly constricted but not so much as those of *D. alopecuri*, and approach those of *C. merceri* much more in general appearance. These larvae may be found in the grass heads in late May and June and again in late July onwards until the following spring.

Pupae. Pupation takes place in the seed case, and about 8 days later emergence takes place. As in *D. alopecuri*, the pupa wriggles up

the seed case until it half protrudes. The cases are easily recognised by the naked eye, as the wing cases, head and thoracic coverings are almost black while the abdominal case is white. When the pupa is full the abdomen is blood red, otherwise the pupa is almost black.

Broods. This species is double brooded, there being a brood on the wing from June to August (summer brood) and another in April and May (spring brood). The dates of emergence of the summer brood vary considerably. The southern and western areas are earlier than the northern and eastern ones. For example, in 1927, the crest of emergence of the summer brood in the Devon sample was on July 1st (the first on June 21st), that in Kent July 4th (first on June 24th), that in Glamorgan July 19th (first on July 13th), that in Rutland July 26th (first on July 20th), and that in Yorkshire July 28th roughly.

Variation in duration of life cycle. The life cycle in the case of the summer brood is about 3–4 months and in the case of the winter brood about 8 months. Just as is the case with *D. alopecuri*, the duration may be considerably lengthened. From a sample of foxtail seed collected August 16th, 1926, in co. Tyrone, midges emerged in June 1927 and then again one male and four females emerged in February 1928. This has not been repeated, although "kept over" material has been used.

7. *Contarinia merceri* n.sp.

(*a*) *History.*

Miss Ormerod, in her eighth report on injurious insects during 1884, deals with midges attacking meadow foxtail seed, and it is at once apparent that besides *D. alopecuri* she was also dealing with another species. In her figure on p. 32 there is the anchor process of *C. tritici* and two differently shaped (pointed and rounded) anchor processes of meadow foxtail midges. The first of these is without the slightest doubt that of *D. alopecuri*, while the second is that of the species at present under consideration. Miss Ormerod was in communication with Mr E. Baillie from the neighbourhood of Chester, and in his letters, quoted by Miss Ormerod, there is ample evidence that he observed the crest of egg laying of *C. merceri* in the evenings ("at eight the field appeared to swarm with them"; cf. the observations under *Oviposition* in this paper). Mr Hunter, also of Chester, was writing to Miss Ormerod at the same date and noted that the larvae crawled out of the heads a day or two after being placed in a box. At this time Miss Ormerod sent the specimens to Mr R. H. Meade, who said they might possibly be small

varieties of *C. tritici*, but that probably it was a new or undescribed species. Only females were sent, this again very strong evidence that they were *C. merceri.*

Since these observations there has been no mention of a *Contarinia* species attacking meadow foxtail grass. But as soon as this investigation was started, a *Contarinia* species was found, and this is presumably the same species.

Experiments have been made to see whether it can mate with *C. tritici* (Kirby) but all the results have been negative. The species is therefore considered to be a distinct species and is described below as *C. merceri* n.sp.

(*b*) *Description.*

This species, which resembles *C. tritici* (Kirby) very closely both in adult structure and larval characters, is named after Prof. S. P. Mercer.

Male. Body length about 1–2 mm. Antennae, 2 + 12, first and second flagellar segments fused, each flagellar segment consisting of two subglobular nodes separated by a stem and each, except the terminal one, with a neck; the stem and neck on any one segment being about equal in length and diameter; each node with a whorl of seven to nine looped regular circumfila; the stem and neck of the 3rd flagellar segment about two to three times as long as broad, those of 10th three to four times as long as broad; distal node of 12th segment bearing a roundly cylindrical setose elongation about as long as neck of 12th segment, setae on all nodes but not stems or necks. Palps: four segments all with moderately short setae, basal segment quadrate, 2nd and 3rd elongated, slightly narrowed at each end, the 3rd slightly longer than 2nd, distal segment rounded, cylindrical, slightly longer than 3rd. Face pale yellow. Eyes black. Thorax brown, lighter at point of wing attachment. Wings clear. Abdomen deep chrome to cadmium yellow (Ridgway). Legs same colour with dark hairs; claws simple, moderately curved, about as long as empodium. Genitalia: basal clasp segment stout; terminal clasp segment with minute setae, short, stout and curved; dorsal plate large, V-shaped emargination, lobes rounded, truncate; ventral plate large, slightly longer than dorsal plate, deep U-shaped emargination, lobes smoothly rounded; style longer than ventral plate, pointed. Co-types: Cecid. 822, 880, 883, 1201, 1202 and 1204.

Female. Length slightly longer than male. Antennae, 2 + 12, normal *Contarinia* female type, neck stout, short on basal segments, longer distally. Palps: four segments, proximal segment quadrate, 2nd, 3rd

and 4th elongate, round, each longer than preceding one. Thorax brown. Abdomen bright deep chrome to cadmium yellow. Ovipositor very long, aciculate, retractile. Otherwise about as in male. Co-types: Cecid. 619–21, 636–40, 642 and 881.

Larva. Deep chrome to cadmium yellow. Lives in flower heads of *A. pratensis* for a few weeks in summer, then "jumps" to the soil, where it remains semiquiescent until the following spring. Causes "blindness" of the grass.

(*c*) *Distribution.*

C. merceri is most abundant and widespread, occurring throughout England (see Table I). It also is to be found in the Isle of Man; Glamorganshire, Cardiganshire and Caernarvonshire in Wales; near Aberdeen in Scotland; in counties Antrim, Armagh, Tyrone, Dublin, Wicklow and Wexford in Ireland. It also occurred in one sample from Finland.

(*d*) *Bionomics.*

Emergence. This species emerges later in the day than either of the two preceding species. The males again emerge slightly before the females and in this species the crest of the emergence of the males is just before noon while that of the females is just after noon. The following figures dealing with the emergences of 644 males and 1776 females support this:

	By noon				After noon			
	Males		Females		Males		Females	
	Actual No.	%	Actual No.	%	Actual No.	%	Actual No.	%
Fourteen samples	418	65	589	33	226	35	1187	67

The date of emergence of the adults in the spring varies considerably. In Hertfordshire in 1928 the earliest emergence noted occurred on May 24th and the latest on July 14th, while the maximum number of emergences occurred on June 20th. In 1927 the crest appeared to be June 8th to 11th, while in 1929 about June 13th.

Besides fluctuating with the season, the date of emergence varies with the locality. For example, large numbers of midges have been observed laying eggs in co. Dublin on May 28th, 1927, Belfast June 9th, 1926, and in Caernarvonshire on June 5th, 1927.

Sex ratio. Out of 2495 emergences in 1928 from English samples, there were 601 males and 1894 females, giving a ratio of 24 males to

76 females. Again, out of 1097 emergences from Welsh and Irish samples, there were 256 males and 841 females, giving a ratio of 23 males to 77 females. Confirmation of this has been obtained from emergences in pots of counted 1000 larvae in 1929. This abnormally low percentage of males is paralleled in parthenogenetic Hymenoptera and may be a feature of the gall-midge genus *Contarinia, e.g. tritici*. Differential sex mortality during development may account for the abnormally low numbers of males.

Mating. Coition lasts about 80 seconds. There is nothing about mating that differs essentially from most other midges.

Oviposition. The females insert their very long needle-like ovipositors right in between the glumes covering the ovary. They do not pierce any sheath. Several eggs are laid in one floret. Oviposition takes place from 7.30 a.m. to noon, a pause round 1.30 p.m., going on again from 4.30 to 9 p.m. There is a crest of egg laying about 7.30 p.m. (all sun time).

Duration of egg stage. The young larvae hatched from the eggs in about 4 days.

Larva. The larvae are deep chrome to cadmium yellow (Ridgway). In other terms, they are golden yellow. In shape they are long and move freely about, usually, however, staying in one floret until they have completed their growth. Then they crawl out of the floret and "jump" to the ground where they remain quiescent until the following spring. They have been observed crawling about on the surface the following May. So-called "blindness" of meadow foxtail grass is due to the damage caused by the larvae.

The larvae of this species do not, as a rule, occur singly in the florets, up to 15 larvae being found in a single floret, in this respect being similar to those of *C. tritici* (Kirby) on wheat, and distinctly different to those of *D. alopecuri* and *S. geniculati.* In 348 florets which were attacked by *C. merceri*, 99 florets contained 1 larvae, 84 contained 2, 58 contained 3, 34 contained 4, 27 contained 5, 13 contained 6, 6 contained 7, 13 contained 8, 9 contained 9, 3 contained 10, 1 contained 11 and 1 floret contained 15 larvae. It is owing to the habit of these larvae migrating to the soil when fully fed that many florets are found to contain only one or two larvae.

Pupa. Pupation takes place in the soil, and it is impossible to collect pupae of this species being certain that no other species is involved, as so many species of *Contarinia* are to be found in the soil and no satisfactory method of separating the pupae of the different species exists. Pupation takes place just over a week before the adult midge emerges

in the spring. By keeping the larvae it is possible to obtain the pupa, and it is found to be slightly darker in the wing cases, head and thorax than in the abdomen. When the midge is ready to emerge, the pupa wriggles to the surface of the soil.

Broods. This species is single brooded as a rule, but in some localities seems to have a second generation. The life-cycle may be briefly summarised as follows: adult midges May to June; larvae growing in size in florets, about 3 to 5 weeks, about June; developing larvae in soil, July to the following May; pupae, May. Thus it will be seen that this species has one brood a year. In 1927 in samples from three localities there seemed to be a partial second emergence. On August 1st adults emerged from a Devon sample (20. vi. 27), from two Hertfordshire samples adults emerged from September 9th to October 29th and from a Leicestershire sample (11. vii. 27) adults emerged from September 15th to October 9th. Again in 1929 females emerged from a Bedfordshire sample (6. vii. 29) in August. It is not thought that this means there is a regular second brood. However, it cannot be ignored as insectary emergences appear from observations to coincide with those in the field. In this species the first spring emergence occurred on the same day in 1928 as midges were observed first in the field, and in the case of *D. alopecuri* the insectary first emergence was only 6 days before the first midges were seen in the field.

Enemies. The Empid fly, *Empis caudatula* Linn. (kindly identified by Mr J. E. Collin), has been observed catching and eating ovipositing females of this species on several occasions.

8. *Lestodiplosis* spp.

At least one species of the genus *Lestodiplosis* lives, in its larval stages, predacious on the larvae of *C. merceri*. There may also be a second species involved. These midges will be described in a subsequent paper dealing with the parasites and predators of midges injurious to meadow foxtail grass. *Lestodiplosis* sp. living in foxtail heads have been found in samples from Shropshire, Nottinghamshire, Lincolnshire, Leicestershire, Caernarvonshire; and co. Wicklow, co. Dublin, co. Antrim. The *Lestodiplosis* occurs far too occasionally to be of any use in controlling *C. merceri.*

9. Parasites.

Parasites are so common that if a sample of seed infested with midges is received from anywhere in England parasites will in all probability emerge. Unfortunately, it has not yet been possible to get these

parasites named except one, which Dr Waterston kindly determined as *Prosactogaster attenuata* Hal. There are several species of Chalcidoidea which are parasitic on these midges. It is curious that *D. alopecuri* seems to suffer very considerably from parasites, while a few parasites have been bred from *C. merceri* but none from *S. geniculati* in British samples. The numbers of host to parasite varies in different years and this is being especially studied over a period of years. Reference may be made to Tables II–V for further information as to numbers of parasites and hosts from different localities.

10. Relative infestation and frequency of the different species of midge.

The three midges, *D. alopecuri*, *S. geniculati* and *C. merceri* are very common and occur in such numbers as to be a serious menace to the growing of foxtail grass for seed. Tullgren (1917) records finding about 57,000 larvae to the pound of seed, in this case *S. geniculati* on marsh foxtail, while a sample of Danish grown meadow foxtail seed received from Dr Dorph Petersen was estimated to have 38,000 seeds per kg. attacked by *D. alopecuri*.

Tables II–V show how abundant these midges are sometimes in Great Britain. For example, from a sample of 72 heads of meadow foxtail collected in Rutland in 1927, 2422 midges and 672 parasites were reared; from a Devonshire sample of 164 heads 2983 specimens of *S. geniculati* alone were reared, while there were hundreds of *C. merceri* larvae present in addition, although only two were reared; from a Belfast sample of 69 heads 1554 specimens of *Dasyneura alopecuri* and 1862 parasites of this species were reared.

In the tables the actual figures of midges and parasites are given, while the number of florets is based on a count of ten heads chosen at random from the sample. In the last column (Tables II–V) are figures representing the percentage number of florets attacked; it is needless to point out that the figures do not represent the infestation, say in any one county, but are merely figures for the particular samples. The figures obtained show at least how numerous the midges might become, if any factor affecting their biology were to change. More than one parasite may emerge from one midge larvae, but here each parasite has been considered to emerge from a separate larva. This is allowable owing to the fact that the maximum emergence of the larvae present did not take place. All the figures, for this reason, are too low. Whether or not there is the same mortality in the field it is hard to

determine exactly. Of hundreds of parasite larvae seen, never more than one has been found in a single floret and no sign has been seen of polyembryony.

That these figures of emergence are really too low is shown by the fact that in pots of 1000 *D. alopecuri* larvae 75–84 per cent. emerged, while in pots of 1000 *C. merceri* larvae only 15–17 per cent. emerged. It has always been far more difficult to rear larvae which remain throughout the winter in the ground than those which winter in some kind of gall or seed case. It is for this reason that in the tables the numbers of *C. merceri* are small, in reality the larvae are present in far larger numbers than either those of *D. alopecuri* or *S. geniculati*.

The enormous number of larvae that are sometimes present may be exemplified as follows. In a sample of 10 heads of the grass chosen at random out of 250 heads collected near Aberdeen (25. vii. 28) and containing 2595 florets, only 18 or 3·8 per cent. ($\pm$ 1·8) florets contained seeds, while 1115 or 43 per cent. ($\pm$ 10·1) were empty, due largely to attack by *C. merceri*, and 1382 or 53 per cent. ($\pm$ 9·2) contained larvae of *D. alopecuri*. Again, out of 1005 florets from 6 heads of grass gathered at random at Rothamsted (11. vii. 28), 356 seeds were formed while 276 florets were empty, 25 contained larvae of *D. alopecuri* or of its parasites, and 348 contained larvae of *C. merceri*. Since in this latter species more than one larva are usually found in a floret, these 348 florets attacked by *C. merceri* contained 1073 larvae. The large number of empty florets is certainly to a large extent due to the fact that some of the larvae had already (by 11. vii. 28) migrated to the soil. Again, in a Dorset sample of 120 heads (26. vi. 29) there were 21,072 larvae of *C. merceri* present; and in a Somerset sample of 50 heads (1. vii. 29) there were 6529 larvae of *C. merceri* present.

Table II.

Summary of emergences, 1927–8.

Locality	No. of heads and florets	Total midges bred	Total parasites bred	*Dasyneura* ♂	*Dasyneura* ♀	*Contarinia* ♂	*Contarinia* ♀	*Stenodiplosis* ♂	*Stenodiplosis* ♀	*Lestodiplosis* ♂	*Lestodiplosis* ♀
England	1,600 391,974	14,176	6,194	3105	4114	640	1994	2009	2306	2	6
Wales	641 124,583	555	69	78	39	70	263	43	62	—	—
Scotland	106 30,422	1,588	979	806	782	—	1	—	—	—	—
Ireland	764 179,093	5,134	6,299	1826	2491	186	578	10	35	2	4
Total	3,111 726,072	21,453	13,541	5815	7426	896	2836	2062	2403	4	10

Table III.

Résumé of foxtail insectary pots, 1927–8.

A. English samples.

Locality and date	No. of heads and florets	Total midges bred	Total parasites bred	*Dasyneura* ♂	*Dasyneura* ♀	*Contarinia* ♂	*Contarinia* ♀	*Stenodiplosis* ♂	*Stenodiplosis* ♀	*Lestodiplosis* ♂	*Lestodiplosis* ♀	In 100 florets
Shropshire (13. vii. 27)	206 41,612	803	1931	79	239	90	285	58* 3	47*	—	1* 1	7
Rutland (20. vii. 27)	72 13,608	2,422	672	543 1*	755 —	27	60	440*	596*	—	—	23
Yorkshire (12. vii. 27)	64 15,296	1,500	464	213	168	260	856	2*	1*	—	—	13
Derbyshire (13. vii. 27)	67 15,276	377	1	115	144	39	79	—	—	—	—	2
Kent (13. vi. 27)	100 24,300	70	92	14 1*	40 —	5	10	(49)*	(80)*	—	—	0·6
Kent (15. viii. 27)	62 19,096	1	1	—	1	—	—	—	—	—	—	0·01
Nottingham (20. vii. 27)	72 18,648	951	1056	350	496	33	52	7*	12*	—	1*	11
Berkshire (8. vii. 27)	233 65,706	301	282	29	85	44	142	—	1*	—	—	0·9
Norfolk (1. vii. 27)	10 1,760	102	—	69	33	—	—	—	—	—	—	6
Lincoln (20. vii. 27)	67 15,142	1,142	673	468	429	21	73	44*	104*	1	1 1*	12
Devon (21. vi. 27)	164 46,412	2,985	—	—	—	1*	1*	1444*	1539*	—	—	6
Leicester (12. vii. 27)	69 22,839	2,177	870	762	1006	— 81	5* 319	— —	3* —	1* —	— —	13
Northumberland (8. vii. 27)	41 10,701	121	33	31	73	—	12	4* —	1* —	—	—	1
Total	310,396	12,952	6075	2675	3469	601	1894	2002	2304	2	5	Infected

* Emerged in 1927.

Table IV.

Résumé of foxtail insectary pots, 1927–8.

B. Welsh samples.

Locality and date	No. of heads and florets	Total midges bred	Total parasites bred	*Dasyneura* ♂	*Dasyneura* ♀	*Contarinia* ♂	*Contarinia* ♀	*Stenodiplosis* ♂	*Stenodiplosis* ♀	*Lestodiplosis* ♂	*Lestodiplosis* ♀	In 100 florets
Caernarvon (16. vii. 27)	465 81,375	224	7	1	3	52	168	—	—	—	—	0·2
Cardigan (8. vii. 27)	76 21,508	86	1	11	—	14	61	—	—	—	—	0·4
Glamorgan (13. vii. 27)	100 21,700	245	61	66	36	4	34	43*	62*	—	—	1
Total	124,583	555	69	78	39	70	263	43	62	—	—	Infected

C. Scotland.

Locality and date	No. of heads and florets	Total midges bred	Total parasites bred	*Dasyneura* ♂	*Dasyneura* ♀	*Contarinia* ♂	*Contarinia* ♀	*Stenodiplosis* ♂	*Stenodiplosis* ♀	*Lestodiplosis* ♂	*Lestodiplosis* ♀	In 100 florets
Aberdeen (27. vii. 27)	106 30,422	1588	979	806	782	—	1	—	—	—	—	8

* Emerged in 1927.

Table V.

Résumé of foxtail insectary pots, 1927–8.

D. Irish samples.

Locality and date	No. of heads and florets	Total midges bred	Total parasites bred	*Dasyneura* ♂	*Dasyneura* ♀	*Contarinia* ♂	*Contarinia* ♀	*Stenodiplosis* ♂	*Stenodiplosis* ♀	*Lestodiplosis* ♂	*Lestodiplosis* ♀	In 100 florets
Co. Armagh (28. vii. 27)	88 15,488	280	679	125	135	1	4	1* 1	13	—	—	6
Co. Tyrone (30. vii. 27)	83 22,908	440	1074	204	201	3	2	8	22	—	—	7
Co. Wicklow (16. vii. 27)	98 20,482	307	23	19	41	43	202	[1 ♂* another species]		—	1*	2
Co. Dublin (13. vii. 27)	98 16,464	917	46	213	206	134	362	—	—	1*	1*	6
Co. Wexford (vii. 27)	80 16,080	70	46	20	49	—	1	—	—	—	—	0·7
Belfast T.T. (18. vii. 27)	69 12,351	1525	2560	575	938	5	5	—	—	—	2*	33
Belfast Swan. Expt. (19. vii. 27)	66 25,740	26	8	7	16	—	2	—	—	1*	—	0·1
Belfast cultiv. (11. viii. 27)	92 24,380	1554	1862	657	897	—	—	—	—	—	—	14
Belfast meadow (9. viii. 27)	90 25,200	15	1	6	8	—	—	[1 ♀ another species]		—	—	0·06
Total	179,093	5134	6299	1826	2491	186	578	10	35 [+2 other species]	2	4	Infected

* Emerged in 1927.

11. Size of meadow foxtail grass heads in different localities.

The number of florets in a head of meadow foxtail grass varies very considerably. In order to ascertain a rough idea of this variation, ten heads were chosen at random from the different samples obtained and the number of florets in each head was counted. Table VI shows the average number of florets for ten heads together with the maximum and minimum numbers.

Since meadow foxtail grows wild and is also cultivated, it is to be expected that plants grown experimentally would have larger heads than indigenous plants. The following figures represent two samples grown experimentally and two indigenous samples:

Belfast "cultivated" (19. vii. 27): av. 390 (max. 536; min. 267)
,, (11. viii. 27): ,, 265 (,, 345; ,, 147)
"wild" (18. vii. 27): av. 179 (max. 209; min. 117)
,, (9. viii. 27): ,, 280 (,, 447; ,, 192)

It is reasonable to suppose that "wild" heads reaching the flowering stage in April would be smaller than those reaching the same stage in May to June. In the case of plants grown under experimental conditions, this difference could be very easily lessened, if not nullified, by the treatment they received. In this connection Prof. Mercer tells me that a smaller percentage of florets appearing early in the year develop into seeds than those appearing later.

Table VI.

Showing number of florets per head.

Locality	Average florets per 10 heads	Maximum	Minimum
Devon (21. vi. 27)	283	388	143
Kent: (*a*) (14. vi. 27)	(*a*) 243	(*a*) 354	(*a*) 180
(*b*) (15. viii. 27)	(*b*) 308	(*b*) 437	(*b*) 243
Leicester (12. vii. 27)	331	519	152
Shropshire (13. vii. 27)	202	359	154
Nottingham (20. vii. 27)	259	339	179
Berkshire (8. vii. 27)	282	380	173
Rutland (20. vii. 27)	189	244	97
Lincoln (20. vii. 27)	226	332	175
Norfolk (1. vii. 27)	176	199	154
Derby (13. vii. 27)	228	382	147
Yorks (12. vii. 27)	239	299	192
Newcastle (8. vii. 27)	261	334	194
Caernarvon (16. vii. 27)	175	224	88
Aberystwyth (8. vii. 27)	283	445	148
Glamorgan (13. vii. 27)	218	340	116
Isle of Man (29. viii. 28)	163	228	88
Orkney (2. ix. 27)	209	307	156
Aberdeen (27. vii. 27)	287	448	221
Co. Wicklow (16. vii. 27)	209	310	145
Co. Wexford (vii. 27)	201	330	106
Co. Dublin (13. vii. 27)	168	230	89
Co. Armagh (28. vii. 27)	176	241	138
Co. Tyrone (30. vii. 27)	276	371	178
Belfast Toxic Tom (27. vii. 27)	179	209	117
Belfast meadow (9. viii. 27)	280	447	192
Belfast (11. viii. 27)	265	345	147
Belfast Exp. Plot (27. vii. 27)	390	536	267

12. Effect of manuring on midges and grass.

It might be supposed that manuring would affect the incidence of midge attack for two reasons; firstly, the grass might be caused to flower at different dates and so not exactly coincide with the normal period of oviposition of the midges; secondly, the constituents in the

florets might be altered and so affect the oviposition and development of the larvae.

In this connection the classical grass plots at Rothamsted were examined. The plots[1] at Rothamsted have been manured in the same way for a number of years and this has affected the flora to a remarkable degree. Dr Brenchley (1924) showed how some types of manuring, *e.g.* nitrate of soda and organic manures, encourage the growth of meadow foxtail, while others, *e.g.* heavy dressings of ammonium salts except in absence of potash or presence of silicates, starved soils and most incomplete manures, do not encourage it. Further, the grass responds to a plentiful supply of nutrients providing a sufficiency of lime is applied. "It evidently needs an abundant nitrogen supply, but cannot take advantage of it if the soil is too acid, and the encouragement by lime is very marked." The accompanying Table VII shows the emergence of midges and parasites from various plots. The numbers of the larvae present in the florets on June 23rd, 1927, were estimated as follows:

Plot 1:	4	per cent. florets contained larvae.
7:	46	,, ,,
13:	13	,, ,,
13 +:	28	,, ,,
17:	6	,, ,,

As *C. merceri* larvae had "jumped" by this date, only the larvae of *D. alopecuri* and *S. geniculati* are taken into account. It will be seen that these estimated larval percentages agree partially with the emergence percentages (Table VII), due allowance being made for a very high larval mortality. The increased mortality in these samples compared with the other samples can be explained by the fact that the samples were kept dry until January 1928 and then put in insectary pots, whereas the other samples were placed in insectary pots as soon as they were received.

Table VIII shows the numbers of florets in heads on different plots.

It will be seen that on the dung and fish meal plot (13) the heads were largest. From the table it would appear that the size of the head may be changed with the manuring.

[1] Plot 1 is treated each year with 206 lb. of sulphate of ammonia per acre; Plot 7 with complete minerals, *i.e.* 3½ cwt. superphosphate, 500 lb. sulphate of potash, 100 lb. sulphate of soda and 100 lb. of sulphate of magnesia per acre; Plot 13 with 14 tons dung alternating with 6 cwt. fish meal per acre, dressings every 2 years; Plot 13 + the same, with the addition of lime at the rate of 2500 lb. ground lime per acre every 4 years; and Plot 17 is treated with 275 lb. nitrate of soda per acre each year.

Table VII.

Résumé of foxtail insectary pots, 1927–8.

A. English samples II. Rothamsted grass plots (23. vi. 27).

Plot	No. of heads and florets	Total midges bred	Total parasites bred	*Dasyneura* ♂	*Dasyneura* ♀	*Contarinia* ♂	*Contarinia* ♀	*Stenodiplosis* ♂	*Stenodiplosis* ♀	*Lestodiplosis* ♂	*Lestodiplosis* ♀	In 100 florets
1	15 1,350	5	—	5	—	—	—	—	—	—	—	0·4
7	88 20,108	785	40	246	532	—	1	6*	—	—	—	4
13	90 23,520	71	9	19	28	2* 2	12* in 25 heads 6	—	1*	—	1*	0·3
13+	90 20,400	312	71	124	70	4* 31	34* in 25 heads 47	1*	1*	—	—	2
17	90 16,200	51	—	36	15	—	—	—	—	—	—	0·3
	81,578	1224	120	430	645	39	100	7	2	—	1	Infected

* Emerged in 1927.

Table VIII.

Showing number of florets per head in different plots.

Plot	Number of florets per head: Average	Maximum	Minimum
1	170	308	90
7	216	293	153
13	294	500	200
13+	255	313	198
17	180	213	128

It is interesting to note that the heaviest attack did not occur on the plot with the largest grass heads, *i.e.* most florets on which to oviposit, nor on the plot with the largest number of heads.

13. Control.

Spraying for the control of these midges may be considered out of the question, since if this were to be done in the winter the spray would have to penetrate the seed cases in order to reach two species *D. alopecuri* and *S. geniculati*, and a soil fumigant would be necessary in order to kill the larvae of *C. merceri*; further, in the summer it would not be practical to spray the growing grass heads either with a deterrent or poison.

A control by a chemotropic substance, or even fires lighted in the evenings to attract the *C. merceri* females, might be possible. The attraction to bonfires has been worked with some success in the case of the

wheat midge, *C. tritici* (Kirby), a species with similar egg laying habits. Chemotropism offers a field for research.

Although the species *D. alopecuri* is heavily parasitised, any practical application of biological control does not appear to be possible. In the case of the two other midge species, parasites are scarce and might be of some help. Spiders, a fly and an Anthocorid have been observed to capture egg laying females, but in spite of such agencies as these the number of midges remains very high.

A simple method of reducing the numbers of midges is offered. Prevent the flowering of the grass until after the bulk of the midges have emerged by allowing sheep to graze on the grass until a certain determined date. Then close down the field. Such a control safety date is used in connection with Hessian fly attack in North America. The date for closing down the field would have to vary with the locality, and this would have to be the result of careful biological observations over a number of seasons. The idea of keeping sheep on the grass resembles closely the feeding of sheep on prematurely advanced winter oats ("winter proud") in some districts in the south-west of England.

In order to test this plan of delaying the flowering of the grass, duplicate plots of grass land were kept cut in 1929 until certain dates and then allowed to grow to seed. Samples of 25 heads of grass were picked from each plot on June 28th, 1929, by which time in that year the crest of emergence of the midges was past about a fortnight. The plots were in Mr Morland's home apiary at Rothamsted and were known to be heavily attacked by *D. alopecuri* and *C. merceri*, while *S. geniculati* was present, but in smaller numbers. As this latter species is double brooded, the first brood being early in the year, any control dates for the other two species would work for this midge.

The results of the trial may be tabulated: in each case 25 heads cut on June 28th were examined. Plots 2 and 3 cut until May 27th and June 10th respectively did not produce any foxtail heads.

Table IX.

Showing result of examination of 25 heads from duplicated plots.

Plot	Treatment	Clean heads	*D. alopecuri* present	*C. merceri* present
1	Control not cut	1	20	23
1 A	,,	0	22	17
4	Cut on April 15th and 29th	7	14	16
4 A	,, ,,	7	16	16
5	Cut on April 15th and May 13th	12	9	7
5 A	,, ,,	11		10

It will be seen that the number of heads infested with midge larvae is reduced by allowing the grass to come to seed later.

In these figures an infected head means one in which at least one midge larva was found but gives no idea of the number present, *i.e.* seeds destroyed, which may be anything from 1 upwards.

In order to examine this more critically the florets of 25 heads were mixed together and then subdivided until a heap of roughly 500 florets was left. The result of this test is shown:

Plot	No. of florets	No. of florets containing larvae	Florets attacked %
1	571	458	80
4	541	239	44
5	546	60	11

This merely confirms the statement that grass allowed to mature after a safety date is cleaner than grass which ripens normally.

One serious drawback to this method of control is that the grass heads which ripen later are smaller, and therefore contain less seeds than grass ripening normally. This, however, might be improved, if it were worth while, by selecting and plant breeding. In the above experiment the extra cleanness of seed more than compensated for the reduction in size of head.

This method of control should be studied more in detail, several points being obscure. For example, the date of closing down the crop would vary in different districts both with the dates of midge emergences and also soil conditions. The latter difficulty might be overcome by the judicious application of artificials. Cockayne (1916), however, stated that mowing and stocking with cattle has been tried in New Zealand without much success; he also added that it might be successful if the life-histories of the midges were properly known.

Two methods of killing the larvae in the seed cases have been worked out by Rostrup (1919), (1) dry heating for 35 minutes to a temperature of 59°–60° C., and (2) treatment with carbon bisulphide (1 gm. CS_2 to a litre air) in a sealed room for 9 hours. Keeping over the seed for a year is also recommended by her.

14. Summary.

1. Three midges do serious damage to the seeding of meadow foxtail grass; they are *Dasyneura alopecuri* (Reuter), *Stenodiplosis geniculati* Reuter and *Contarinia merceri* n.sp. All three occur almost wherever the grass is grown.

2. The distribution and bionomics of these midges are dealt with; *D. alopecuri* has one brood a year, *S. geniculati* has two, while *C. merceri* usually has one but occasionally may have two.

3. "Blindness" or empty husks in meadow foxtail grass is due very largely to attacks of *C. merceri*, which midge does the most extended damage.

4. Keys are given for the separation of larvae, pupae and adults.

5. Control measures are discussed and a method of keeping sheep on the grass until a certain safety date, *i.e.* a date when the crest of emergence of the female midges is over, is strongly advocated in districts where the bionomics is known.

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INSECTS FOUND ASSOCIATED WITH CACAO, SPICES AND DRIED FRUITS IN LONDON WAREHOUSES

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(With Plates XXIII–XXXII.)

Introduction.

The present paper is an account of the insects found attacking or in association with stored products in London warehouses. Our attention was mainly directed towards pests of cacao, spices and dried fruit; cereals, which would harbour a large number of additional pests, have not yet been examined. The survey was carried out in conjunction with Mr W. S. Thomson, of the Empire Marketing Board, as part of a study of the pests of stored products undertaken by the Department of Entomology of the Imperial College of Science and Technology, financed by the Empire Marketing Board. Mr Thomson's report on the economic importance of insect infestation has recently appeared (Munro and Thomson, 1929). Plates XXVIII and XXIX are reproduced from Munro and Thomson's report, by kind permission of H.M. Stationery Office, to whom we are indebted for the loan of the blocks.

In the identification of the insects and arachnids we have received much kind help from the following gentlemen: Coleoptera, Mr K. G. Blair, except Staphylinidae which were identified by Dr Malcolm Cameron; Lepidoptera, Mr W. H. T. Tams; Hymenoptera, Dr J. Waterston and Mr Claude Morley; Diptera, Mr F. W. Edwards and Mr J. E. Collin; Hemiptera, Mr W. E. China; Orthoptera, Mr B. P. Uvarov; Copeognatha, Mr J. V. Pearman; Arachnids, Mr W. S. Bristowe. Commdr. J. J. Walker kindly supplied us with references to the occurrence of *Araecerus* in England. We are also grateful to the gentlemen who have allowed us to have access to their warehouses. We have not recorded the particular warehouses in which pests were captured, but all the records are from London. Our work has been done under the direction of Dr J. W. Munro, of the Department of Entomology, to whom we are indebted for much encouragement, advice and criticism.

In the following account the pests are arranged systematically, the orders succeeding each other approximately according to their importance. For each species, besides our own food records for adults and larvae, we have added a certain number of references to the literature to indicate the range of food and the distribution. We have made no attempt to give a complete bibliography of any one species. In certain cases, where it appeared necessary, we have added notes of a systematic nature.

COLEOPTERA.

Carabidae.

Plochionus pallens F. Adult found in Grenada nutmegs. Has been imported into Rouen and Marseilles, Ganglbauer (1892, p. 410). Distribution: Africa, E. Indies, N. and S. America.

Staphylinidae.

Atheta coriaria Kr. Adult found in fermented Jamaica green ginger. Oct. 11th, 1927. Perhaps preying on the larvae of the fly, *Scatopse*. Recorded from the sap of felled trees, Fowler (1888, p. 110). Distribution: cosmopolitan.

Atheta trinotata Kr. Probably has the same habits as the preceding. Recorded from decaying vegetable matter, Fowler (1888, p. 107). Distribution: Europe.

Atheta nigricornis Thoms. Adult found in very old and much insect-damaged Afghan sultanas. Recorded from sap, fungi and decaying vegetable matter, Fowler (1888, p. 113). Distribution: Europe.

Philonthus sordidus Grav. Adult bred from larva found in fermented Jamaica green ginger. Oct. 11th, 1927. Perhaps preying on the larvae of the fly, *Scatopse*. Recorded from dung and haystack refuse, Fowler (1888, p. 268). Distribution: Europe.

Nitidulidae.

Carpophilus dimidiatus F. Adult found in cacao (Costa Rica, Ecuador, Panama, Venezuela, Gold Coast); nutmegs (Grenada, Straits Settlements); ginger (Jamaica); sultanas (Afghanistan). Larva found especially in nutmegs; also cacao and ginger. Adult occurs all the year round. In Bermuda the adult also occurs in the open on various fruits, Ogilvie (1928). Zacher (1929) records it from the following products: dried fruit, dates, cacao, wheat, drugs, and earth-nuts. Distribution: cosmopolitan.

Carpophilus hemipterus L. (Fig. 1). Adult found in Smyrna figs; Australian dried pears; also windows of fruit warehouses. Larva found in Smyrna figs. A common pest of dried and fresh fruits, Essig (1915), Myers (1928). Distribution: cosmopolitan.

Carpophilus ligneus Murray. Adult found on the windows of a fruit warehouse, and of one where cacao and sugar are stored. Blair (1922) records it from: Californian dried plums (Liverpool); dried apples (Penarth); jelly blocks (London); also from Birmingham and Isle of Wight. Prunes and pears in Berlin (Zacher, 1929). Murray (1864, p. 351) described it from Mexico. Distribution: N. America and Germany (Zacher).

Carpophilus flavipes Murray. Adult found between the double sacks which enclose Indian gum damar; the sacks had probably held grain just previously. Described by Murray (1864 p. 359) from Celebes and Singapore.

Zacher (1929) records several other species of *Carpophilus* found in stored products in Germany. The species which we have found may be distinguished as follows:

1. Posterior angles of the thorax completely rounded. Thorax (Fig. 2) flattened, shining, with finer punctures. Second antennal joint about as long as third **Carpophilus ligneus** Murray

   Posterior angles of the thorax obtuse but distinct. Thorax more coarsely punctured 2

2. Second joint of the antennae (Fig. 3) distinctly shorter than the third. Blackish brown species, legs and antennae red-brown, elytra brown, darker at the scutellum and at apex, especially laterally. (Unusually dark or pale specimens are not rare) **Carpophilus dimidiatus** F.

   Second joint of the antennae slightly or distinctly longer than the third. Club of antennae usually darker than the funicle 3

3. Elytra with the shoulders and a large apical area pale yellow. Second joint of the antennae (Fig. 4) distinctly longer than the third **Carpophilus hemipterus** L.

   Pale markings of elytra, when present, red-brown 4

4. Black to very dark brown species with the shoulders of the elytra red-brown. Second antennal joint distinctly longer than the third **Carpophilus flavipes** Murray

   Black-brown species with the anterior margin of the thorax, the shoulders, the outer third, suture and apex of the elytra, red-brown. Second joint of the antennae hardly longer than the third . . . . **C.** sp.

The unidentified *Carpophilus* was found in West African cacao. Mr K. G. Blair tells us that it does not agree exactly with any species in the British Museum collection. It appears to be nearest to *C. mutilatus* Er.

We have found the larvae of *C. hemipterus* and *C. dimidiatus*. Zacher (1929) figures the larva of the former. They may be distinguished by the degree of separation of the anal processes. In *C. hemipterus* (Fig. 5) the emargination between the processes is nearly twice as wide as a process, while in *C. dimidiatus* (Fig. 6) it is narrower than one of the processes.

TROGOSITIDAE.

Tenebroides mauretanicus L. (Fig. 7). Adult found in Grenada nutmegs; cacao (Trinidad, Samoa, Gold Coast); Australian currants and sultanas. Larva common in nutmegs, occasional in cacao. Occurs in many stored products, see Back and Cotton (1926 *a*). Distribution: cosmopolitan.

Tenebroides oblongus Shp. We found two dead adult specimens in Samoan cacao; it was described by Sharp (1891, p. 423) from two specimens. Distribution: Panama, Mexico.

The two species of *Tenebroides* differ as follows:

Size larger; length about 10 mm.; colour black-brown; eighth joint of antennae (Fig. 8) forming part of the club. Apex of the scutellum rounded

Tenebroides mauretanicus

Size smaller, length 6–7 mm.; colour red-brown; eighth joint of antennae (Fig. 9) resembling the seventh, not forming part of the club; scutellum sharply pointed

Tenebroides oblongus Shp.

Lophocateres pusillus Klug. Adult found on windows of cacao and sugar warehouse. First recorded as British by Newbery (1918) from butter-beans in London. Roeppke (1926) records it from rice in Java. Distribution: cosmopolitan.

LATHRIDIIDAE.

Enicmus minutus F. Adult found on damp walls of warehouses. Not found in any product; see Wallace (1921) for an account of these beetles swarming on the walls of a house. Distribution: cosmopolitan.

Lathridius bergrothi Reitt. Adult found on walls of warehouses. *Lathridius bergrothi* is a comparatively recent introduction, which has now become common (Thornley, 1901). Distribution: Europe.

Corticaria fulva Com. Adult found in floor sweepings of a cacao and spice warehouse. Fowler (1913, p. 263) records this species from a corn-shop, a granary and from wine-cellars. Distribution: cosmopolitan.

CUCUJIDAE.

Ahasuerus (*Cathartus*) *advena* Waltl. Adult found in cacao (Gold Coast, Cameroons, Grenada, St Lucia, Panama, Ecuador, Samoa); Jamaica ginger; Grenada nutmegs. Found in cacao stores in Germany by Zacher (1926 *b*). Strong (1921 *b*, 1922 *a*), and Maskew and Strong (1920, p. 724) record this species from bulbs, herbs, algaroba pods, yams, pine nuts and maize. Distribution: cosmopolitan.

Cathartus (*Silvanus*) *cassiae* Reiche. (*C. gemellatus* Duv., *C. quadricollis* Guér.) Adults found on cacao (Gold Coast); also walls of a sugar and cacao warehouse. For synonymy see Zacher (1927 *a*). Often found in maize and other cereals in N. America, cf. Strong (1921 *a*, p. 331), Morrill (1917). First British record Durrant (1921, p. 34). Zacher (1926 *a*) states that the fruits of *Cassia fistula* are the natural food. Distribution: Europe, America, Africa.

Laemophloeus ferrugineus Steph. Adult found in currants (Greece); cacao (Gold Coast); chillies (Mombasa); between the double sacks enclosing Indian gum damar. Fowler (1889, p. 298; 1913, p. 263) in granaries, also sometimes out of doors under bark or in wasps' nests. It appears to be most usually found in grain, *e.g.* Hewitt (1920, p. 16) records it from Australian wheat in Canada. Distribution: cosmopolitan.

Laemophloeus turcicus Grouv. (Fig. 10). Adult found in cacao (West Africa); chillies (Mombasa); nutmegs (Grenada). Joy (1924) first recorded this species from England, having found specimens at Twyford, Berks. in a flour-mill. It is probably widespread. Grouvelle (1877) described it from specimens found in Turkish figs. Distribution: Europe and the Orient.

Laemophloeus minutus Ol. (*pusillus* Schönh.). Adult found in nutmegs (Grenada); cacao (Ceylon, Gold Coast); between the double sacks of Indian gum damar; also on walls, etc. of warehouses. Larva found in nutmegs (Grenada). Fowler (1889, p. 298)

records it from granaries; Linnaniemi (1920), Argentine maize; Strong (1922 *a*), bulbs; Morstatt (1914, p. 3), cotton seed; Hargreaves (1924), overripe coffee-berries and fruits of wild Rubiaceae in Uganda; Rye (1871), filberts. Distribution: cosmopolitan.

Laemophloeus janeti Grouv. One adult found in cacao (West Africa). Described by Grouvelle (1899, p. 177) from a specimen in West African cacao in Paris; recently the biology in the Congo has been described by Mayné (1916) and Ghesquière (1922). Distribution: India, Madagascar, West Africa (Grouvelle, 1908, p. 464).

Key to the species of *Laemophloeus* recorded above:

1. Antennae (Fig. 11) as long as or longer than the body, the last joint very elongate, about five times longer than broad, the two preceding joints more than three times longer than broad. Head rather strongly and closely punctured . . . **Laemophloeus turcicus** Grouv. Male
   Antennae less elongate 2
2. Antennae (Fig. 12) about two-thirds as long as the body, the last joint about four times as long as broad, the two preceding joints two and a half times as long as broad. Punctuation of the head as in *L. turcicus*. Thorax more rectangular, less narrowed behind than the other species. Size small . . . **Laemophloeus minutus** Ol. Male
   Antennae (Fig. 13) much shorter, about half as long as the body, or less, the last joint about twice as long as broad, two preceding joints about one and a half times as long as broad 3
3. Mandibles with a blunt tooth (Fig. 14) on the lower side towards the base. Head shining, finely and sparsely punctured; head large, thorax considerably narrowed behind . . . . **Laemophloeus ferrugineus** Steph. Male
   Mandibles simple. Head smaller, thorax less narrowed behind 4
4. Size larger, more elongate. Head shining, with smaller, relatively sparse punctures 5
   Size smaller, less elongate. Head duller, with more numerous and larger punctures 6
5. Thorax less shining, more finely punctured; side keel of thorax less developed; hind angles of thorax less produced. Scutellum strongly transverse. Elytra duller . . . . **Laemophloeus ferrugineus** Steph. Female
   Thorax more shining, but what punctures there are, larger; side keel of thorax strong throughout. Hind angles of thorax produced into fine points. Scutellum triangular. Elytra relatively smooth and shining . . . **Laemophloeus janeti** Grouv. (? Sex)
6. Size larger, head more strongly and closely punctured. Thorax more narrowed behind . . . . **Laemophloeus turcicus** Grouv. Female
   Size smaller, head less strongly and closely punctured, though more so than *L. ferrugineus*. Thorax more rectangular, less narrowed behind . . . **Laemophloeus minutus** Ol. Female

It is almost impossible to separate particular specimens of the females of *L. pusillus* and *L. turcicus*.

The species here identified as *L. janeti* Grouv. agrees very well with

the original description, but the identity cannot be regarded as absolutely certain without comparison with authoritative material. Our specimen is perhaps a male.

Oryzaephilus (*Silvanus*) *surinamensis* L. (Fig. 15). Adult found in sultanas (Greece, Smyrna, Australia); currants (Greece); raisins (Australia); dates (Mesopotamia); dried pears (Australia); mace (Grenada); cacao (St Lucia, Grenada, Samoa). Larva found in Australian sultanas. A widespread and omnivorous pest, see Back and Cotton (1926 *b*). Distribution: cosmopolitan.

Oryzaephilus (*S.*) *mercator* Fauv. Adult found in nutmegs (Grenada); chillies (Mombasa); cacao (St Lucia, Samoa, Gold Coast); sultanas (Smyrna); also in almonds and dates of unknown source. Larva found in nutmegs (Grenada). First recorded from England by Tomlin (1905). Fauvel (1889), Guillebeau (1890) and Zacher (1926 *b*) record it from arachis, palm fruits, coconut fibre, granaries, corn-mills and cacao stores. Distribution: N. America, Europe.

There has been some misapprehension as to the secondary sexual characters of the male of *O. mercator* Fauvel. Fowler (1913, p. 124), Champion (1896) and Guillebeau (1890) all state that the hind femur of the male of this species lacks the tooth found in the male of *O. surinamensis* L. In reality this tooth, as well as one on the posterior trochanter, is present in the male of both species. They differ in the length of the temples behind the eye. In *O. mercator* (Fig. 16) the temples are less than half the vertical diameter of the eye, while in *O. surinamensis* (Fig. 17) they are well over half this distance.

Cryptophagidae.

Cryptophagus saginatus Stm. Adult found in Afghan sultanas. Fowler (1889, p. 318) records it as common in warehouses and also in the open in decaying vegetable matter. Distribution: N. America, Europe.

Cryptophagus scanicus L. (Fig. 18). Adult found in Afghan sultanas; Smyrna figs; Australian dried pears. Larva found in Afghan sultanas. Fowler (1889, p. 319) records it from vegetable refuse in the open and commonly from houses. Distribution: Europe.

Var. *patruelis* Stm. Adult found in Afghan sultanas. The variety occurs with the type but is much rarer.

Cryptophagus validus Hbst. Adults found in sultanas (Australia, Afghanistan); also damp walls of warehouses. Larva found in Afghan sultanas. Fowler (1889, p. 321) records it from decaying vegetable refuse, sometimes in warehouses. Distribution: Europe, especially the south.

Cryptophagus dentatus Hbst. Adult found in Afghan sultanas. Fowler (1889, p. 321) records it from decaying vegetable refuse, and Wollaston (1871) from a granary in Madeira. Distribution: Europe.

Cryptophagus distinguendus Stm. Adult found on walls of a warehouse. Fowler (1913, p. 265) records this beetle from granaries and bakeries; also in the open. Distribution: Europe.

Cryptophagus acutangulus Gyll. Found with the preceding species. Fowler (1889, p. 322) records it from decaying vegetable refuse and Newbery (1912) from a London warehouse. Distribution: Europe, N. America.

Cryptophagus cellaris Scop. Adult found in Australian sultanas; also common on damp walls of warehouses. Fowler (1889, p. 323) records it from decaying vegetable refuse and cellars; Keys (1920), from bread. Distribution: Europe, N. Africa, Turkestan, N. America.

Cryptophagus affinis Stm. Adult found in Australian sultanas. Fowler (1889, p. 324) records it from decaying vegetable refuse; Wollaston (1871) from a granary in Madeira. Distribution: Europe.

Henoticus californicus Mann. (*germanicus* Reitt.). Adult found in a cacao and spice warehouse. First recorded from a London warehouse by Newbery (1912); also found in dried fruit in California by Parker (1915), who recorded it as *H. serratus* Gyll.; see also Champion (1912) for synonymy. Also found in jam, Blair (1920); corks, Newbery (1912) and Blair (1920); bread, Keys (1920). Distribution: Germany, France (Falcoz, 1915, p. 91), England, Holland. Probably a native of America.

MYCETOPHAGIDAE.

Typhaea stercoraria L. (*fumata* L.). Adult found in cacao and spice warehouse. Fowler (1889, p. 349) records it from granary refuse. Distribution: Europe, N. America.

DERMESTIDAE.

Dermestes lardarius L. (Fig. 19). Adult found in cacao (W. Africa, Ecuador, Jamaica, St Lucia); nutmegs (Grenada); dried pears (Australia); between the double sacks enclosing Indian gum damar; also common in warehouses. Larva found in Areca nuts (? India); cacao (Ecuador); dried pears (Australia); also common in warehouses. Larvae probably often eating exuviae of other insects, parasitised larvae of *Ephestia*, etc., rather than the product in which they are found. Fowler (1889, p. 358) records it from warehouses. Saunders (1866) found the larva on hides; Zacher (1926 *b*) found it in a cacao-store and O'Mahony (1928) found it damaging tobacco; Sacharov (1921) records it damaging dried fish; Kreyenberg (1928) has studied the life history.

Dermestes cadaverinus F. Adult found in cacao (West Africa); ginger (West Indies); spice warehouse. Larva found in cacao (West Africa); Illingworth (1916) found it eating dead cockroaches in Hawaii, while Strong (1922 *b*, p. 775) found it in dried mushrooms. Kimura and Takakura (1919) record it as a pest of dried fish. Distribution: cosmopolitan.

Dermestes vulpinus F. Adult found in cacao (Ecuador, Venezuela, Costa Rica, St Lucia); also on some African cowries. Larva found in cacao (Venezuela); African cowries. Most often recorded from hides—Fowler (1889, p. 357), Jones (1889)—also damages tobacco (O'Mahony, 1928) and many other products. Sometimes a serious pest of dried fish, Illingworth (1918). According to Reh (1927) damage to products other than hides is due only to penetration for pupation. Kreyenberg (1928) has studied the life history. Distribution: cosmopolitan.

Dermestes frischi Kug. Adult found in cacao and spice warehouse; on cowries from Africa. Fowler (1889, p. 357) records it from carrion; Zacher (1926 *b*) found it

in a cacao warehouse and Hamilton (1884) found it damaging dried fish. Distribution: Europe, Asia, N. America.

Dermestes carnivorus F. Adult found in cacao (Costa Rica). According to Fauvel (1889) it is often imported into France with hides from La Plata. Distribution: cosmopolitan.

As appears from the above list, several species of *Dermestes* occur in stored products which are not included by Fowler (1889, p. 356) in his account of the British species. Newbery (1913) has recorded another species, *D. peruvianus* Casteln., introduced at Liverpool. The following key, partly based on Ganglbauer's (1904), distinguishes these warehouse species.

1. Anterior half of the elytra pale brown with a small undulated black mark in the centre of each pale area . . . . **Dermestes lardarius** L.
   Elytra unicolorous, black or, when immature, reddish brown 2
2. Eyes normal (Fig. 20), as in *D. lardarius*, not so convex as to be hemispherical. Sides of the thorax for about a quarter of its width with obvious patches of white hairs which are directed inwards 3
   Eyes large (Fig. 21), fully hemispherical. Sides of thorax without such obvious hair-patches, what hairs there are being directed backwards 5
3. Apex of each elytron produced into a fine point. White hairs absent from a small round spot near the hind angles of the thorax . . **Dermestes vulpinus** F.
   Apex of each elytra not produced. No black spot in the lateral hair-patches 4
4. Antennal club (Fig. 22) pitchy red, narrower, less compact; seventh joint not laterally produced, eighth only slightly so. Metasternum and second abdominal sternite with dense fine punctures and a few scattered much larger ones. Male with small tufts of yellow hairs arising from minute depressions in the centre of the disc of the third and fourth abdominal sternites **Dermestes carnivorus** F.
   Antennal club (Fig. 23) black, broader, more compact. Punctures of the mesosternum and second abdominal sternite larger and of uniform size. Male with a small tuft of hairs on the fourth sternite only . . **Dermestes frischi** Kug.
5. The impressed lateral lines of the first abdominal sternite strongly curved outwards, arising at the outer corner of the epimeron of the metathorax. Male with a small tuft of yellow hairs in the centre of the disc of the fourth abdominal sternite . . . . **Dermestes peruvianus** Casteln.
   The impressed lateral lines of the first abdominal sternite more curved inwards, arising at the inner corner of the epimeron of the metathorax or in the centre of the hind margin. In the male both the third and fourth abdominal sternites with tufts of yellow hairs . . . . . **Dermestes cadaverinus** F.

The *Dermestes* larvae which have so far been obtained in the survey are those of *D. lardarius* L., *D. cadaverinus* F. and *D. vulpinus* F.

They may be most easily distinguished by means of the spines on the dorsal surface of the penultimate abdominal segment.

1. Spines (Fig. 24) nearly at right angles to the body, curved, placed close together as in a V, the actual tips directed backwards; no pronounced median bristle **Dermestes lardarius L.**
2. Spines (Fig. 25) sloping to the rear, straight, with a definite space between their bases; no pronounced median bristle . . . . **Dermestes cadaverinus F.**
3. Spines (Fig. 26) sloping to the rear, recurved, set moderately close together, with the actual tips directed forwards, a pronounced curved median bristle placed slightly behind them . . . . **Dermestes vulpinus F.**

Karsch (1887) gives a key to separate a number of *Dermestes* larvae. Rey (1886, 1889) describes those of *D. cadaverinus* F. and *D. vulpinus* F. in great detail. Ganglbauer (1904) gives further references.

Attagenus pellio L. Adult found on windows of warehouses. Fowler (1889, p. 359) states that it is a common household insect. Distribution: cosmopolitan.

Anthrenus verbasci L. (*varius* F.). Adult found in cacao (Ecuador); also on windows, etc. of a cacao and spice warehouse. Fowler (1889, p. 363) records it attacking natural history specimens and Felt (1919) found it breeding in maize. Distribution: cosmopolitan.

Helocerus fuscus Ol. (*claviger* Er.). Adult found in cacao and spice warehouse. A household insect, also recorded by Fowler (1889, p. 364) from flowers in the open. Distribution: Europe, N. America.

SCARABAEIDAE.

Cyclocephala tetrica Voet. One dead adult found in nutmegs (Grenada). Distribution: Jamaica.

Diplognatha silacea Moel. One dead adult found in coffee (? origin). Distribution: S.E. and Central Africa and Angola.

Phileurus didymus L. One dead adult found in nutmegs (Grenada). Distribution: S. America.

CLERIDAE.

Corynetes analis Kl. One living adult found in cacao and spice warehouse; ? West African cacao. Distribution: Africa.

Necrobia rufipes De G. Adult found in cacao (W. Africa, Samoa); nutmeg (Grenada); figs (Smyrna); African cowries; also common in warehouses. Larva found in cacao (West Africa, Samoa). Probably often spreads into the Samoan cacao from the copra on the same ship. Recorded from cacao by Zacher (1926 *b*) but a more important pest of copra, Aders (1916), Roeppke (1926), and Corbett and Dover (1927). Distribution: cosmopolitan.

Thaneroclerus buqueti Lefevr. Adult found in cacao (Ceylon); also occasionally found by miscellaneous collecting in warehouses. Larva found in Ceylon cacao. Recorded by Fowler (1913, p. 280) as occurring in ginger from Bombay and preying on *Lasioderma serricorne* F. and by Keuchenius (1917) preying on the same beetle in tobacco in Java. Distribution: India, imported into Europe.

PTINIDAE.

Gibbium psylloides Czemp. (*scotias* Kl.). Adult found in floor-sweepings of a cacao and spice warehouse. Fowler (1890, p. 185) records it from old houses in decaying refuse; Fletcher and Ghosh (1920), who found it in granaries in India, figure all stages. Lucas (1884) found it in cayenne pepper. Distribution: cosmopolitan.

Niptus hololeucus Fald. Adult found in cacao and spice warehouse. Zacher (1927 *b*) has recently described the omnivorous habits of this species. Since Kolbe (1889) described its spread in Europe, this species has, especially recently, become a more important pest. Distribution: Europe, N. America.

Ptinus fur L. Adult abundant on sacks of ginger (? Jamaica); also common on walls of warehouses. Recorded from West African cacao by Knapp (1921); also a common household insect, Fowler (1890, p. 181). Boieldieu (1856) and Zvierezomb (1917) recorded it from granaries; Miller (1922), from dates; Carpenter (1920), from henbane seed. Distribution: cosmopolitan.

Ptinus tectus Boield. (Fig. 27). Adult found in cacao (W. Africa, Dominica, Grenada); nutmegs (Grenada); almonds (? Spain); ginger (Jamaica); figs (Smyrna); sultanas (Australia, Afghanistan); dried pears (Australia); dried apricots (Australia). Larva found in cacao; dried pears; dried apricots; sultanas. Beare (1904) first recorded this beetle as British, but it had already at that date spread to some extent in England, though not so common as it is now. At the present day it appears to have replaced *P. fur* L. as the commonest British species; Zacher (1927 *a*, p. 112) gives a long list of foods. Distribution: probably native of Tasmania (Champion, 1904); also occurs in Australia and New Zealand, N. America. Has recently reached Germany, Zacher (1922).

Ptinus latro F. Adult found in cacao and sugar warehouse. Fowler (1890, p. 182) records it as very rare in England in houses. Boieldieu (1856) records it from granaries. Distribution: Europe.

Ptinus brunneus Duft. Adult found in a cacao and sugar, and in a dried fruit warehouse. Fowler (1890, p. 181) records it from warehouses and Boieldieu (1856) from granaries. Distribution: Europe, N. Africa, Asia, N. America, and New Caledonia.

Trigonogenius globulum Sol. Adult found in dried pears (Australia). First recorded in England by Tomlin (1900) from flour-mills in Lancashire; Strong (1921 *a*, p. 332) found it in vegetable ivory (*Phytelephas*); Scott (1921) records it breeding in argol. Distribution: native of Chile; Germany and America.

Anobiidae.

Anobium punctatum De G. Adult not rare in the woodwork of warehouses. Larva found in woodwork. The well-known furniture beetle. Distribution: cosmopolitan.

Sitodrepa (*Anobium*) *panicea* L. Adult found in cacao and spice warehouse; dried fruit warehouse. Zacher (1926 *b*) records it from a cacao warehouse in Germany. Fowler (1890, p. 191; 1913, p. 280) gives as foods old bread, flour, lettuce seeds, ginger, dead insects and vermicelli. Den Doop (1917) records it from coriander and caraway seed. Mokrzecki (1916) found it a pest of stored grain. Macgillavry (1907) records it from strychnine. Distribution: cosmopolitan.

Catorama herbarium Gorh. Adult found in nutmegs (Grenada); also on windows, etc. of cacao and spice warehouse. Dash (1917) and de Faria (1919) record this beetle attacking books and furniture in Barbados and Brazil, respectively. Fisher (1920, p. 62) found it damaging brooms in the Panama canal zone. Distribution: S. and C. America, West Indies.

Lasioderma serricorne F. Adult found in cacao (West Africa); nutmegs (Grenada); ginger (Jamaica). Larva found in ginger. Most notorious as a pest of tobacco, Runner (1919). Fowler (1890, p. 195) records it from ginger and liquorice; Maskew and Strong (1920, p. 724) from aniseed, pumpkins and tamarind seeds; Den Doop (1917) from coriander and caraway seeds. Distribution: cosmopolitan.

BOSTRYCHIDAE.

Dinoderus minutus F. Adult found in ginger (Jamaica); cacao and spice warehouse. Fowler (1913, p. 149) records it from roots, and cotton. De Charmoy (1915, p. 7) found it attacking maize in Mauritius. Lesne (1924, p. 66) also lists the woods that are attacked. Distribution: tropicopolitan and introduced into most of the temperate regions.

LYCTIDAE.

Lyctus brunneus Steph. Adult found in an old log lying in a cacao and spice warehouse. Not a pest of the products dealt with in the present paper. Lesne (1924, p. 84) summarises its habits. Distribution: Europe, Japan, Madeira, Africa, West Indies.

CISSIDAE.

Cis pygmaeus Marsh. Adult found in decayed green ginger (Jamaica). Usually found—Fowler (1890, p. 210)—on fungi on trees. Distribution: Europe.

TENEBRIONIDAE.

Alphitobius diaperinus Pz. Adult found in cacao (Ceylon); between the double sacks enclosing Indian gum damar. The synonymy of this and the following species is so confused that it is almost impossible to assign records to one or the other species. Both, however, appear to occur as minor pests in a variety of products, *e.g.* cereals, linseed, cotton seed, cacao, chocolate, ground nuts and tobacco. Distribution: cosmopolitan.

Alphitobius laevigatus F. Adult found in cacao (Trinidad). See the remarks under the preceding species. Distribution: cosmopolitan.

Gnathocerus cornutus F. Adult found in a cacao and spice warehouse and in ginger (Jamaica). Recorded by Fowler (1891, p. 20; 1913, p. 295) from flour, meal and bread. Traizet (1895) also found it very abundant in a bakery. Shepherd (1924) and Morison (1925) describe the life history, and the latter shows that it thrives best in products already infested by other insects. Distribution: cosmopolitan.

Gnathocerus maxillosus F. Adult found in nutmegs (? origin) and in a cacao and spice warehouse. First British record, Durrant (1921, p. 34), from maize. Maskew and Strong (1920, p. 724) found it in pumpkin and tamarind seeds. Distribution: France, Africa, Madeira, N. America.

Tribolium castaneum Herbst. (*T. ferrugineum* auctt. nec. F.) (Fig. 28). For the synonymy of this species see Blair (1913). Adult found in cacao (W. Africa, St Lucia, Trinidad, Grenada, Panama, Costa Rica, Venezuela, Ecuador, Ceylon, Samoa); nutmegs (Grenada, Straits Settlements); chillies (Mombasa); almonds (Palestine); seed tapioca (Batavia); sultanas (Afghanistan); figs (Smyrna); sacks of rubber (? origin); between the double sacking enclosing Indian gum damar. Larva found in nutmegs (Grenada). Adult much the commonest in the summer months. The life history has been described by Fletcher (1914). Recorded from a great variety of products: stored cereals, Fletcher and Ghosh (1920); bran and flour, de Charmoy

(1915, p. 12); lentils, Dupont (1916); butter-beans, Newbery (1918); arachis, Roubaud (1917, p. 54); dried fruit, Myers (1928); lac, Imms and Chatterjee (1915, p. 37). Distribution: cosmopolitan.

Tribolium confusum Duv. Adult found in cacao (St Lucia). It is recorded from beans and wheat, Strong (1922 *b*, p. 776); rice, corn, biscuits and cashew nuts, Bodkin (1916); ground nuts, Roubaud, (1917, p. 54); cacao warehouse, Zacher (1926 *b*). Girault (1912) records further foods. Distribution: cosmopolitan.

Fowler's key (1891, p. 18) separates only the males of *Gnathocerus* and *Tribolium*. The following key is available for both sexes, and includes *G. maxillosus* F., not recorded by Fowler.

1. Mandibles with a large tooth directed upwards. Clypeus anteriorly deeply sinuate opposite this tooth **2**
   Mandibles without a tooth. Clypeus rounded anteriorly **3**
2. Mandibular teeth much narrower at the apex than at the base, where each tooth is about as broad as the distance between them. Clypeus produced into a rounded projection between the teeth (Fig. 29). Length 4–4½ mm.
   Gnathocerus cornutus F. Male
   Mandibular teeth narrower, of the same width almost to the apex; at the base each tooth is about one-third as broad as the distance between them. Clypeus produced into a truncate, subquadrangular projection between the teeth (Fig. 30). Length 3–3½ mm. . . . . . . **Gnathocerus maxillosus** F. Male
3. Clypeus more finely and sparsely punctured than the vertex, and situated at a lower level. Elytra smoother and more shining **4**
   Clypeus and vertex continuous and equally strongly punctured **5**
4. Thorax fully one and a half times as broad as long. Greatest width of the head is just in front of the eyes (Fig. 31) . . **Gnathocerus cornutus** F. Female
   Thorax (Fig. 32) about one and a quarter times as broad as long, or less. Greatest width of the head is across the eyes . **Gnathocerus maxillosus** F. Female
5. Apical three joints of the antennae forming a club. Vertex not raised into a definite ridge overhanging the eyes . . . **Tribolium castaneum** Hbst.
   Apical three joints of the antennae not forming a distinct club. Vertex raised on either side into ridges overhanging the eyes (Fig. 33)
   Tribolium confusum Duv.

Caenocorse subdepressa Woll. Adult found in cacao (West Africa). Recorded by Fowler (1913, p. 173) from granaries and by Champion (1896) from arachis. In U.S.A. it has been found under bark—Chittenden (1896). Distribution: probably cosmopolitan.

Sitophagus hololeptoides Castln. Adult found in nutmegs (Grenada). Described from Cayenne and imported into Madeira (Wollaston, 1858 *a*) and Marseilles (Mulsant, 1854); in each case being redescribed; see synonymy in Heyden (1906). Distribution: S. America, imported into Madeira and S. Europe.

The genus *Sitophagus* runs down in Fowler's (1891, p. 18) key to the Tenebrionidae, subfamily Ulomina, to *Alphitobius*. *S. hololeptoides*,

however, is a much flatter and rather more shining insect than either of our two species of *Alphitobius*; the explanate margin of the elytra ends abruptly some distance before the apex; the elytral interstices are hardly punctured; the antennae are at least one and a half times as long as the head (in *Alphitobius* about as long as the head).

MONOMMIDAE.

Monomma brunneum Thoms. Adult found dead in a case of papain (Ceylon). Fletcher (1916) found that this species breeds in the rotten stems of the Papaya, the tree from which papain is derived. Distribution: India.

ANTHICIDAE.

Anthicus australis King. One dead adult found in dried pears (Australia). Distribution: Australia.

Anthicus floralis L. One living adult found in cacao (Samoa). Durrant (1921, p. 34) found it in maize. Distribution: cosmopolitan.

ANTHRIBIDAE.

Araecerus fasciculatus De G. (Fig. 34). Adult found in cacao (West Africa, Panama); nutmegs (Grenada, Singapore). Common in nutmegs and West African cacao, rare in West Indian and South American cacao. Larva found in cacao and nutmegs. Day (1908) lists the occurrence of this species in England in the last century. A general account is given by Reh (1907), Tucker (1909) and Munro and Thomson (1929), while Cotton (1921) has described the larva and pupa. It has been found in many products: *Areca catechu*, Beeson (1919); yams, Maskew (1920); sweet potatoes, Seurat (1901); cassia and ginger, Fauvel (1889); nutmeg, Strong (1922 *b*, p. 776); monkey-pod, Bridwell (1920 *a*); coffee, Friederichs (1927); cacao, Knapp (1921); mace, Chittenden (1916, p. 14); strychnine, Macgillavry (1907). Distribution: cosmopolitan.

CURCULIONIDAE.

Caulophilus latinasus Say. Adult found in ginger (Jamaica). Larva found in ginger. Cotton (1922) gives a general account and in (1921) describes the larva and pupa. It feeds on maize, chick-peas, millet, acorns, avocado and, more rarely, the roots of *Colocasia esculenta* and the sweet potato. In Southern U.S.A. it feeds on maize in the open. Distribution: Central America, West Indies, southern U.S.A., and Madeira.

Sitophilus (*Calandra*) *granarius* L. Adult found in currants (Greece); figs (Smyrna). This important cereal pest does little damage to the products dealt with in the present paper. Distribution: cosmopolitan.

Sitophilus (*C.*) *oryzae* L. Adult found in tapioca (Batavia); sacks of rubber (? origin). See preceding species. Distribution: cosmopolitan.

IPIDAE.

Stephanoderes buscki Hopk. Adult found commonly in nutmegs (Grenada), but always dead. Distribution: Described from Trinidad by Hopkins (1915, p. 30).

Dryocaetes sp. Adult found in nutmegs (Grenada).

LEPIDOPTERA.

Phycitidae.

Plodia interpunctella Hb. (Fig. 35). Adult found in sultanas (Australia, Smyrna); currants (Greece); figs (Smyrna); prunes (Cyprus); dried pears (Australia); dried apricots (Australia); almonds (? Spain); raisins (California); larva found in the same products as the adults. Myers (1928) has recently dealt with the life history and habits. Also recorded as feeding on: grain, Girault (1912); pea-nuts, Popenoe (1911); arachis, Roubaud (1917, p. 55); chillies, Strong (1922 *a*); palm-seed, Strong (1921 *a*, p. 334); chestnuts, nougat, Fallou and Ragonot (1871); figs, currants, dried bilberry (*Vaccinium*), almond, seeds of stone-pine and spruce, Sorhagen (1882). Distribution: cosmopolitan.

Ephestia elutella Hb. (Fig. 36). Adult found in cacao (all countries examined); nutmegs (Grenada); sultanas (Australia); prunes (Cyprus). Larvae found in cacao and prunes. The common pest of cacao, see Reh (1907), Zacher (1926 *b*) and Munro and Thomson (1929). Kirby as early as (1884) recorded it attacking cacao in London. Also stated to attack arachis, Mason[1] (1915); flour, stored grain, etc., Mokrzecki (1916); ceratonia beans, Kieffer (1914, p. 535). Many records must be regarded as doubtful owing to the difficulty of identification. Distribution: cosmopolitan.

Ephestia cautella Wlk. Adults found in sultanas (Australia); currants (Greece); figs (Smyrna); nutmegs (Grenada, Strait Settlements); cacao (Grenada, St Lucia, Venezuela, Costa Rica). Larvae found in sultanas, currants, figs, nutmegs. Myers (1928) describes the attacks of this species on dried fruits. Zacher (1926 *b*) records it from a cacao warehouse in Germany. It also attacks arachis and many other foods, Roubaud (1917, p. 56), Chittenden (1911). Many of the records must be regarded as doubtful owing to the difficulty of identification. Distribution: cosmopolitan.

Ephestia kühniella Zell. Adults apparently very rarely found in cacao warehouses but a pest of cereals and cereal products. Burkhardt (1920) has recently dealt with the life history. Knapp (1921) states, possibly as a result of mis-identification, that it is the commonest species in cacao warehouses in England; according to Zacher (1926 *b*) the larva will only feed on cacao if bred up on it from the egg. Sheppard (1926) records it from dry chillies and beans, and Dieuzeide (1926) states that potatoes, cacao and biscuits are attacked in storage. Distribution: cosmopolitan.

Myelois ceratoniae Zell. Adult found in a dried fruit warehouse where perhaps it had emerged from Smyrna figs. Recorded from shelled almonds, Sheppard (1926); Robinia seeds, walnuts and chestnuts (*Castanea*), Lounsbury (1919); navel oranges, Anon (1926, p. 197); fruits of *Erythrina monosperma*, Swezey (1923); carob (*Ceratonia*), dates, raisins, dried figs, fruits of *Cydonia japonica* and dried insects, De Stefani (1919). Distribution: Europe, Syria, Africa, South America.

We have made a study of the genitalia of the species of *Ephestia* recorded above. We propose to deal with these structures in greater detail on another occasion, but some brief notes are necessary here to indicate what we mean by the names we have employed. We have

[1] In Mason's report (1915) the larva feeding on arachis was merely identified as a Pyzalid. The adult was identified at the Imperial Bureau as *E. cautella* Wlk., but a wrong specific name was given when the identification was published in the *Review of Appl. Ent.* This misnomer was repeated by Munro and Thomson (1929, p. 22).

not yet been able to examine the types of any of the species but we have proceeded on the following assumptions. The most important *Ephestia* attacking cacao is *E. elutella* Hb. After examining about a hundred specimens either caught where cacao was stored or bred from it, and finding that nearly all the specimens have one type of genitalia, we have called moths with genitalia of this type, *E. elutella*. In the same way *E. kühniella* Zell. is mainly attached to cereal products; this species, however, is distinguishable by its larger size, elongate wings, and by the particular shade of grey of the wings alone. Finally an *Ephestia* which we have found in dried fruit warehouses, in nutmegs and more rarely in cacao, we have called *E. cautella* Walk. We have bred a species allied to but apparently distinct from *E. cautella* from some Cameroon cacao. This species we have not yet identified with any previously described *Ephestia*. In Figs. 42 and 46 we have illustrated the genitalia of the male and female of *Plodia* for comparison.

Males.

1. Dorsal edge of the clasper without a tooth-like process
   Ephestia elutella Hb. (Fig. 38)
   Dorsal edge with a tooth-like process 2
2. This tooth consists merely of a production, near the apex of the clasper, of the continuous longitudinal thickening of the dorsal edge. Oedeagus with a narrow, strongly chitinised eversible bar. Tegumen not produced at the apex
   Ephestia kühniella Zell. (Fig. 39)
   The tooth arises before the apex of the longitudinal thickening. Oedeagus with a broad eversible plate. Tegumen produced into a finger-like process on the outer side of the base of each clasper . . . **Ephestia cautella** Wlk. (Fig. 40)

The male of the unidentified species from Cameroon cacao may be distinguished from that of *E. cautella* by means of Figs. 40 and 41.

Females.

1. Eighth sternite more than twice as long as broad. Ovipositor lobes together more than twice as long as broad. Ductus bursae without a longitudinal thickening, bursa with one or two small chitinisations . **Ephestia kühniella** Zell. (Fig. 43)
   Eighth sternite quadrate 2
2. Ovipositor lobes together fully twice as long as broad. Ductus bursae without a longitudinal thickening, bursa with a row of about seven chitinisations .
   Ephestia elutella Hb. (Fig. 44)
   Ovipositor lobes together not much longer than broad. Ductus bursae with a longitudinal thickening, bursa with two to four chitinisations
   Ephestia cautella Wlk. (Fig. 45)

Females bred with the peculiar males from Cameroon cacao differed from ordinary *E. cautella* females in having four chitinisations in the

bursa and in having a broader chitinised band connecting the lateral areas of the eighth tergite; this band also lacked a central, tooth-like projection. These differences, however, are not outside the range of variation of normal *E. cautella*.

Galleriidae.

Corcyra cephalonica Stt. (Fig. 37). Adult found in currants (Greece); nutmegs (Grenada); cacao (Trinidad). Larvae found in nutmegs (Grenada); cacao (W. Africa, Grenada, Trinidad, St Lucia, Venezuela, Ceylon, Samoa). Chittenden (1919) describes the life history; he records attack on rice, cacao, chocolate, ships biscuits and sesame seeds; Roubaud (1916) on arachis; Ritchie (1926) on bullrush millet; Vayssière and Mimeur (1925) on cotton seed. Distribution: Cosmopolitan.

Trachylepidia fructicassiella Rag. Larvae found in *Cassia fistula* pods. Van Emden (1925) found the larva in the same product in Germany. Distribution: India.

Oecophoridae.

Endrosis lactella Schiff. Adult found in warehouses. Meyrick (1928, p. 668) records it as a common house moth, whose larva feeds on dry vegetable refuse. Distribution: Cosmopolitan.

Borkhausenia pseudospretella Stt. Adult found in warehouses. Meyrick (1928, p. 671) records it as the common house-moth, whose larva feeds on seeds, dried plants, insects, skins, etc. Distribution: Cosmopolitan.

Tineidae.

Tinea granella L. Adult found in dried fruit warehouses. Larva found in pistachio nuts (? origin); in a dried fruit warehouse, perhaps breeding in Smyrna figs.

HYMENOPTERA.

Bethylidae.

Holepyris hawaiiensis Ashmd. (Fig. 47). Adult found in cacao (W. Africa, St Lucia, Venezuela, Ceylon); chillies (? origin); also living in refuse in the warehouse. Ashmead (1901) described this species from Hawaii. We have examined the type in the British Museum and find that the legs are entirely yellowish and the wings hyaline. Our specimens, as in Kieffer's (1914) description, have a dark patch on the wings before the apex; they differ from his description, however, in having the four posterior femora dark. Structurally our specimens are so like the type, that we regard them as one species. Bridwell (1920 *b*, p. 311) found that the adult will attack the larvae of *Plodia* and *Ephestia* in captivity; we have also found it would attack larvae of the former moth. Distribution: Hawaii, probably spreading by commerce.

Cephalonomia carinata Kieff. Adult found in cacao (W. Africa, Costa Rica, Ceylon); sultanas (Australia); currants (Greece); chillies (? origin). Described by Kieffer (1907, p. 295) from Albania; Myers (1928 p. 21) has shown that it is a parasite of the beetle, *Oryzaephilus surinamensis* L. Distribution: Australia, England, probably cosmopolitan.

We have also found another Bethylid in Cameroon cacao; in Kieffer's (1914) key it runs down to the genus *Plastanoxus*, but it has bare eyes.

It probably belongs to an undescribed genus, but our material is at present very scanty.

ICHNEUMONIDAE.

Campoplex prytanes Cam. Adult found in dried fruit warehouse and identified by Mr C. Morley. Probably a parasite of *Ephestia*. Distribution: described by Cameron (1903) from Darjeeling. Morley (1913) adds no further records.

BRACONIDAE.

Microbracon (*Habrobracon*) *hebetor* Say. (*brevicornis* auctt. in part) (Fig. 48). Adult found in cacao (all countries examined); sultanas (Australia, Smyrna); currants (Greece); figs (Smyrna); dried pears (Australia). Often very abundant in cacao and sultanas. Adults mainly in the late summer. Larva a parasite of those of *Plodia*, *Ephestia*. The synonymy has been discussed by Cushman (1922) and Muesebeck (1925). It appears to attack exclusively larvae of moths which live in stored products, *e.g. Ephestia* spp. *Plodia*, *Galleria*, *Sitotroga*. Hase (1922) and Myers (1928) have described its habits. Distribution: cosmopolitan.

Doryctes gallicus Reinh. Adult found in dried fruit warehouse. Richards and Thomson (1928). ? Parasite of a wood-boring beetle in fruit boxes. Distribution: France, England.

ENCYRTIDAE.

Zeteticontus laeviscutum Thoms. Adult found in sultanas (Afghanistan). Larva a parasite of full-fed larvae of *Cryptophagus validus* and probably also of *C. scanicus*. Described by Thomson (1876, p. 166) from Sweden, but apparently not since recognised. Distribution: Europe.

DIPTERA.

ANISOPODIDAE.

Anisopus fenestralis Scop. Adult found on the windows of a dried fruit warehouse. This species breeds normally in dust, etc., on floors. Distribution: Europe, N. America.

Anisopus punctatus Mg. Adult found on the windows of a cacao and spice warehouse. Larva found in floor sweepings. Its habits are the same as the preceding species. Distribution: Europe, N. America.

SCATOPSIDAE.

Scatopse fuscipes Mg. Adult found in fermenting green ginger (Jamaica). Larva found with the adult. Larvae have been found in dung and rotting onions. Edwards (1925, p. 274). Distribution: Tasmania, Peru, N. America, Europe.

MYCETOPHILIDAE.

Sciara annulata Mg. Adult found in fermenting green ginger (Jamaica); sultanas (Australia); also on warehouse window. Has been reared from larvae which were damaging cucumbers. Edwards (1924, p. 538). Distribution: Europe.

SCENOPINIDAE.

Scenopinus (*Omphrale*) *fenestralis* L. Adult found on windows of warehouses. Larvae probably feed on those of other insects, see Kröber (1925). Distribution: Europe, N. Africa, N. America, India.

Muscidae.

Calliphora erythrocephala Mg. Adult common on warehouse windows. The common blow-fly. Distribution: cosmopolitan.

Anthomyidae.

Fannia canicularis L. Adult common on warehouse windows. One of the commoner domestic flies. Distribution: cosmopolitan.

Spilogaster uliginosa Fall. Adult found on a box of Greek currants. Chevalier (1924) has shown that the larva feeds on those of such domestic moths as *Borkhausenia*. Distribution: Europe, N. America.

Borboridae.

Limosina sylvatica Mg. Adult found on warehouse windows. Usually associated with manure and decaying vegetable matter. Distribution: Europe and N. America.

Limosina fungicola Hal. Adult found on warehouse windows. Common in decaying vegetable matter; often found on house windows. Distribution: Europe.

Limosina crassimana Hal. Adult found on warehouse windows. Distribution: Europe and America.

Limosina heteroneura Hal. Adults bred from fermented green Jamaica ginger. Larva found in ginger. Distribution: Europe, Africa and Formosa.

Drosophilidae.

Drosophila funebris F. Adult found in figs (Smyrna); also on warehouse windows. The commonest domestic species, breeding in all kinds of decaying animal and vegetable matter. Distribution: Europe, N. America, N. Africa.

Drosophila obscura Fall. Adult found on warehouse window. A fairly common domestic species. Distribution: Europe.

Drosophila melanogaster Mg. Adult found in figs (Smyrna); sultanas (Afghanistan); also warehouse windows. The well-known fruit fly. Distribution: Europe, N. America, Cuba, N. and S. Africa.

Drosophila sp. near *D. repleta* Woll. Adult found in figs (Smyrna).

Drosophila fenestrarum Fall. Adult found on warehouse window. A common domestic species. Distribution: Europe, N. Africa.

Drosophila immigrans Sturt. Adult found on warehouse window. Distribution: N. America, Europe, Formosa.

Scaptomyza tetrasticha Beck. Adult found on warehouse window. Usually an out-of-door species, mining the leaves of Crucifers. Sturtevant (1921), however, records that allied forms, also normally leaf miners, will breed in tomatoes, potato tubers and banana agar. Distribution: Europe.

The following is a key to the Drosophilids mentioned above:

1. Thorax dull grey with the bristles standing in dark spots. Fore tarsi in the male on the inner side with long outstanding hairs, which are longer than the second tarsal joint . . . . **Drosophila sp. near D. repleta** Woll.
   Thorax unicolorous or with continuous stripes. Male front tarsi without these long hairs 2

2. Front femora on the antero-ventral surface with a row of small black bristles. Cross veins and the ends of the second, third and fourth veins irrorated with black **Drosophila immigrans** Sturt.
   Front femora with only the usual long posteroventral bristles. Wings hyaline 3
3. Thorax with only four rows of acrostichal bristles (two rows posteriorly). Facial keel not produced downwards into a nose-like process. One of the bristles at the end of the palpi large and stout, the others small 4
   Thorax with six or more rows of acrostichal bristles. Facial keel nose-like. Bristles at the end of the palpi smaller and at least two of equal size . . 5
4. Yellowish species with a blackish abdomen. Arista of antennae with two long hairs on its ventral surface before the apical fork. Male genitalia large. Male with the first two joints of the anterior tarsi on the inner side with tufts of whitish hairs at the apex . . . . . **Drosophila fenestrarum** Fall.
   Grey species with a yellow head and darker abdomen. Arista ventrally with only one such hair. Male genitalia small, anterior tarsi simple **Scaptomyza tetrasticha** Becker
5. A dull black-brown species with a more shining abdomen. One vibrissa much larger than the others. Medium-sized species . . **Drosophila obscura** Fall.
   At least the pleura pale yellow. Two large vibrissae 6
6. A large brownish species, sometimes with traces of reddish stripes on the thorax. Male anterior tarsi simple . . . . **Drosophila funebris** F.
   A small yellow species with the apex of the abdomen black. Male with a dense comb of black bristles on the inner side of the anterior metatarsi. **Drosophila melanogaster** Mg.

The species allied to *D. repleta* Wollaston (1858 *b*) may be *D. mulleri* Sturtevant (1921), but the latter author does not describe the male secondary sexual characters. One of us (O. W. R.) has examined the types and paratypes of *D. repleta* in the British Museum. The six specimens are all in rather bad condition, having been gummed on cards, and the majority appear to be females. Two of them which may be males lack, as far as can be seen, any ornamentation of the fore tarsi.

D. immigrans Sturtevant, is the species referred to by Duda (1924 *a*) as *D. tripunctata* Becker, nec Loew. Mr J. E. Collin, however, has pointed out to us that the present identification is almost certain.

Duda (1921) has revised the European species of *Scaptomyza.* He unites all the European species having four rows of acrostichal bristles under the name *S. apicalis* Hardy. There are two very distinct forms; one mainly yellow, which he terms var. *flava* Fallen, and one grey with a yellow head, var. *tetrasticha* Beck. The structure of the female ovipositor in these forms is so different that they must certainly be regarded as distinct species. The form we have captured is that figured by Duda (Fig. 7) as var. *tetrasticha* Becker.

There has been much confusion over the name of *D. melanogaster* Mg. Villeneuve (1913) established the present identification on the basis of a Meigen specimen in the Paris Museum. Duda (1924 *a*) examined another Meigen specimen in the Vienna Museum and found that this one was identical with *D. funebris* F. For the present species, therefore, he reverted to the name *D. ampelophila* Lw., which had been in use before the publication of Villeneuve's paper. In view of the large genetical literature which has grown up round the name *D. melanogaster* and because no one can say whether the Paris or the Vienna specimen is really the type, it appears best to follow Villeneuve.

Piophilidae.

Piophila varipes Mg. Adult found on warehouse window. Well known in England. Duda (1924 *b*, pp. 98 and 167) notes that though fond of animal substances, the species also occurs in woods, etc. and has been bred from dead leaves. Distribution: N. and Central Europe, England.

Milichiidae.

Meoneura obscurella Fall. Adult found in Australian sultanas. Zacher (1927 *a*, p. 198) records this species as once abundant in snuff. Distribution: Europe, Egypt.

HEMIPTERA.

Anthocoridae.

Piezostethus flavipes Reut. Adult found in cacao (W. Africa, Grenada, Panama). Young stages sometimes inside cacao beans. Van Emden (1925) found it in roots of *Ipomaea turpethum* from Africa, and Butler (1907; 1923, p. 326) records it from a granary (Carmarthen) in wheat from Basra; macropterous forms rare. Distribution: France, Germany, N. Italy, England.

Lyctocoris campestris F. Adult found on warehouse window. Butler (1923, p. 323) states that it is one of the commonest British bugs. Distribution: cosmopolitan.

ORTHOPTERA.

Blattidae.

Blatta orientalis L. Adult found in wax (Jamaica). The common domestic cockroach. Distribution: cosmopolitan.

Periplaneta americana L. Adult found in nutmegs (Grenada); gum tragacanth (Bushire). Widespread in England under artificial conditions. See Lucas (1920, pp. 104–5). Distribution: cosmopolitan.

Periplaneta australasiae F. Adult found in ginger (Jamaica); gum tragacanth (Bushire); wax (Jamaica). Numerous records in Lucas (1920, pp. 109–11). Distribution: cosmopolitan.

Leucophaea (*Rhyparobia*) *maderae* F. Adult found in ginger (Jamaica). Several records in Lucas (1920, p. 116). Distribution: cosmopolitan.

Blabera Sp. Young. Adult found in ginger (Jamaica). Lucas (1920, p. 117) records two species from England. Distribution: S. and C. America and West Indies.

DERMAPTERA.

LABIIDAE.

Labia arachidis Yers. Adult found in cacao (Grenada, Ceylon); nutmegs (Grenada); ginger (Jamaica). Recorded from various places in England, chiefly in bone-works. See Lucas (1920, pp. 37–8). Distribution: cosmopolitan.

COPEOGNATHA.

TROGIIDAE.

Lepinotus inquilinus Heyd. Adult found in figs (Smyrna); also on floors of a dried fruit warehouse. A domestic species. Distribution: Europe.

Clothilla pulsatoria L. Adult found in sultanas (Afghanistan). A domestic species. Distribution: Europe.

Liposcelis virgulatus Pearman. Adult found in West African cacao. Distribution: ? West Africa.

Chaetopsocus richardsi Pearman. Adult found in West African cacao.

ARACHNIDA.

PHOLCIDAE.

Pholcus phalangioides Fuessl. Adult found in a dried fruit warehouse. Distribution: cosmopolitan.

AGELENIDAE.

Tegenaria parietina Fourcr. The common warehouse spider. Distribution: Europe.

THERIDIIDAE.

Teutana grossa C.L.K. Adult found in a dried fruit warehouse. Distribution: cosmopolitan.

SUMMARY.

1. The pests found in certain cacao, spice and dried fruit warehouses in London have been enumerated. Reference has been made to some of the more important papers in which the food, habits or distribution of the species have been described.

2. To make the identification of some of the pests more easy we have made new keys to the species met with in the following genera: *Carpophilus*, *Laemophlaeus*, *Dermestes*, *Gnathocerus* and allies, *Ephestia* and *Drosophila*.

3. The following insects appear to be recorded from Britain for the first time. Those marked with an asterisk have only been found dead. Coleoptera: *Plochionus pallens* F. (Carabidae), *Carpophilus flavipes* Murr. (Nitidulidae), *Tenebroides oblongus* Shp.* (Trogositidae), *Laemophlaeus janeti* Grouv. (Cucujidae), *Dermestes cadaverinus* F. and *D. carnivorus* F. (Dermestidae), *Cyclocephala tetrica** Voet., *Diplognatha silacea** Moel. and *Phileurus didymus** L. (Scarabaeidae), *Corynetes analis* Kl. (Cleridae), *Catorama herbarium* Gorh. (Anobidae), *Monomma brunneum** Thoms.

(Monommidae), *Sitophagus hololeptoides* Casteln. (Tenebrionidae), *Anthicus australis** Lea (Anthicidae), *Caulophilus latinasus* Say (Curculionidae), *Stephanoderes busckii** Hopk. (Ipidae). Hymenoptera: *Holepyris hawaiiensis* Ashmd. and *Cephalonomia carinata* Kieff. (Bethylidae), *Campoplex prytanes* Cam. (Ichneumonidae), *Zeteticontus laeviscutum* Thoms. (Encyrtidae). Diptera: *Drosophila immigrans* Sturt. (Drosophilidae).

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EXPLANATION OF PLATES XXIII—XXXII

PLATE XXIII.

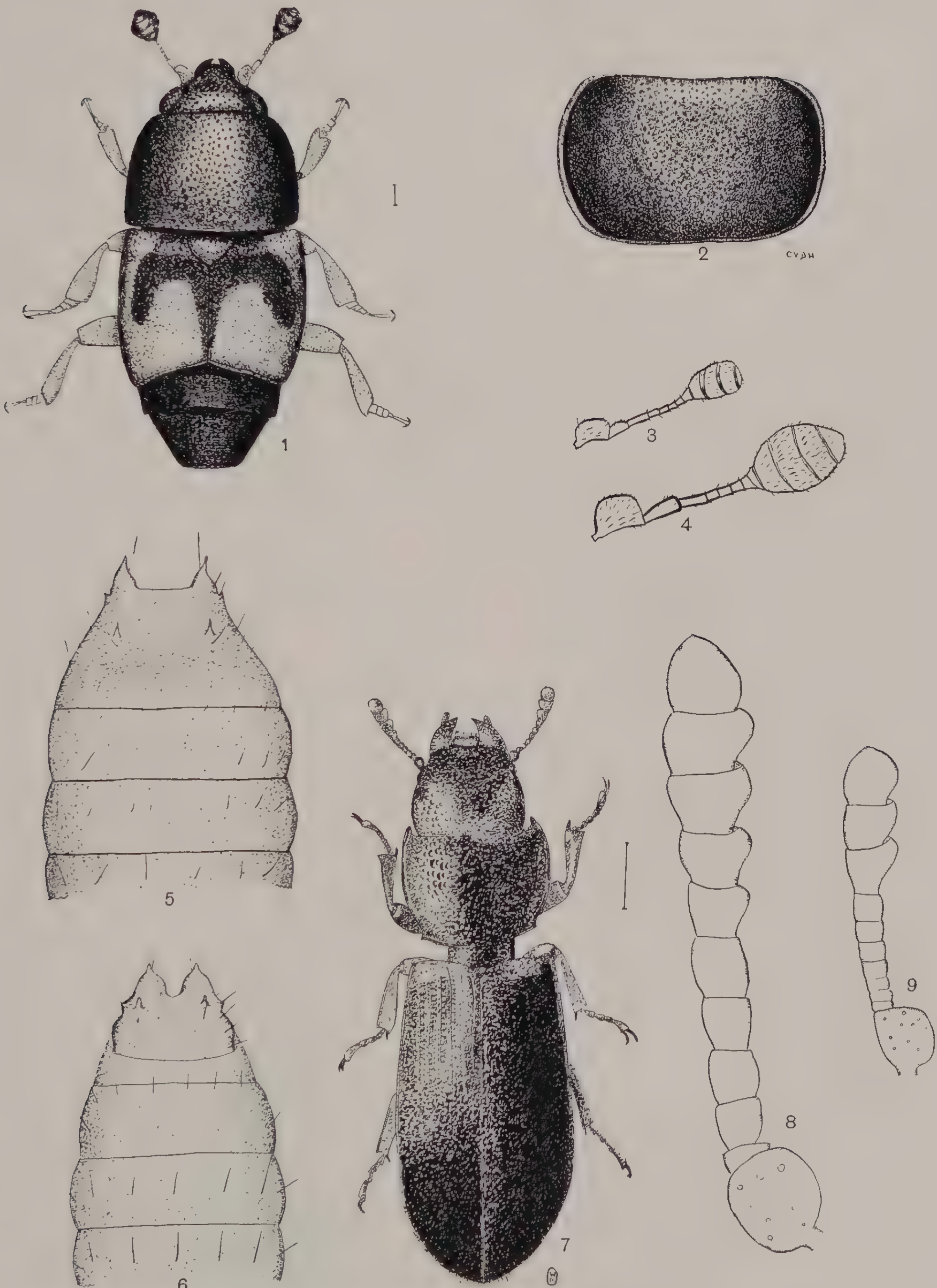
Fig. 1. *Carpophilus hemipterus* L.
Fig. 2. The thorax of *Carpophilus ligneus* Murray.
Fig. 3. The right antenna of *Carpophilus dimidiatus* F.
Fig. 4. The same of *C. hemipterus* L.
Fig. 5. Posterior end of the larva of *Carpophilus hemipterus,* seen from above.
Fig. 6. The same of *C. dimidiatus* F.
Fig. 7. *Tenebroides mauretanicus* L.
Fig. 8. Left antenna of *T. mauretanicus.*
Fig. 9. The same of *T. oblongus* Shp.

PLATE XXIV.

Fig. 10. *Laemophloeus turcicus* Grouv. male.
Fig. 11. Left antenna of *L. turcicus,* male.
Fig. 12. The same of *L. minutus* Ol., male.
Fig. 13. The same of *L. ferrugineus* Steph., male.
Fig. 14. The head of the male of *L. ferrugineus* Steph., seen from in front.
Fig. 15. *Oryzaephilus surinamensis* L., male.
Fig. 16. The head of *O. mercator* Fauv.
Fig. 17. The head of *O. surinamensis* L.

PLATE XXV.

Fig. 18. *Cryptophagus scanicus* L.
Fig. 19. *Dermestes lardarius* L.
Fig. 20. The head of *D. lardarius.*
Fig. 21. The head of *D. cadaverinus* F.
Fig. 22. The right antenna of *D. carnivorus* F.
Fig. 23. The same of *D. frischi* Kug.
Fig. 24. The posterior end of the larva of *D. lardarius,* seen from the left.
Fig. 25. The same of *D. cadaverinus,* seen from above.
Fig. 26. The same of *D. vulpinus,* seen from the left.



RICHARDS & HERFORD.—Insects found with Cacao, etc., in London Warehouses (pp. 367–395).

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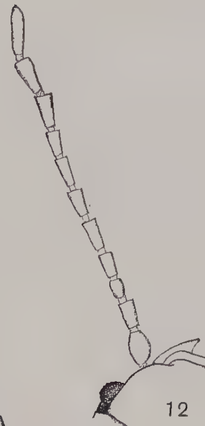

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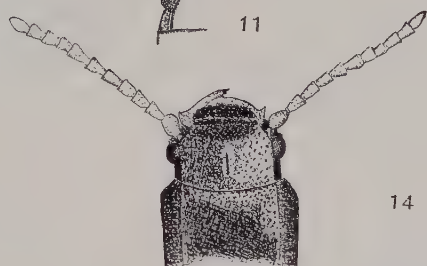

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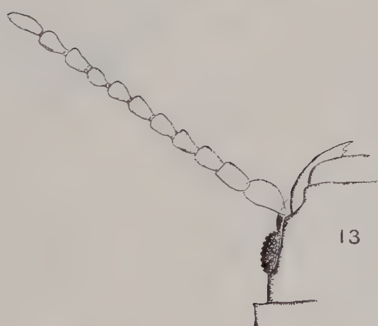

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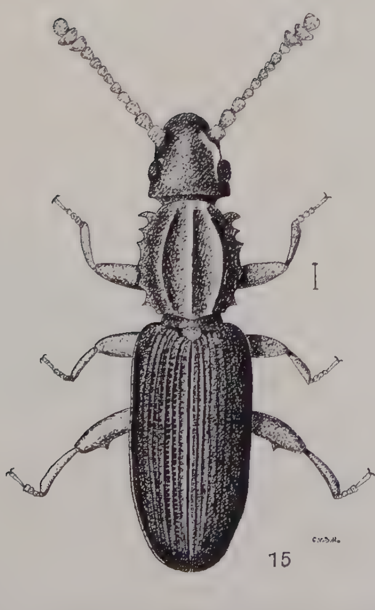

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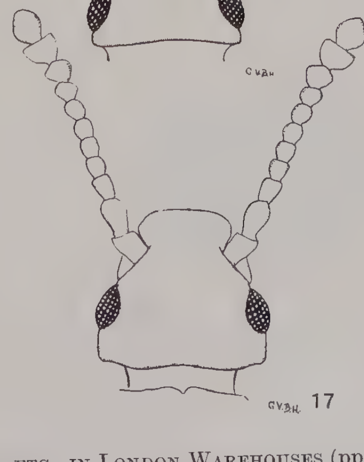

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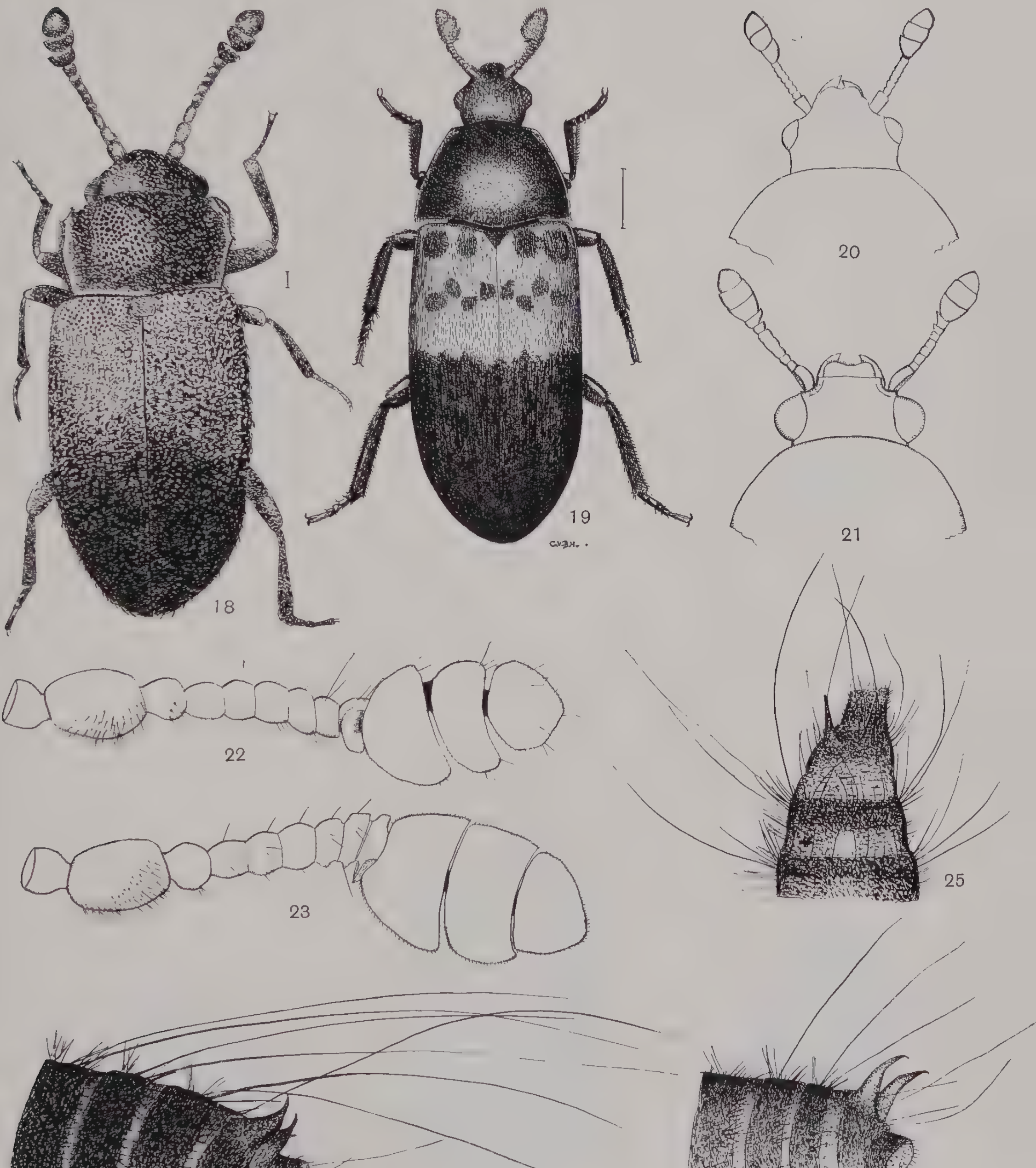
RICHARDS & HERFORD.—Insects found with Cacao, etc., in London Warehouses (pp. 367–395).



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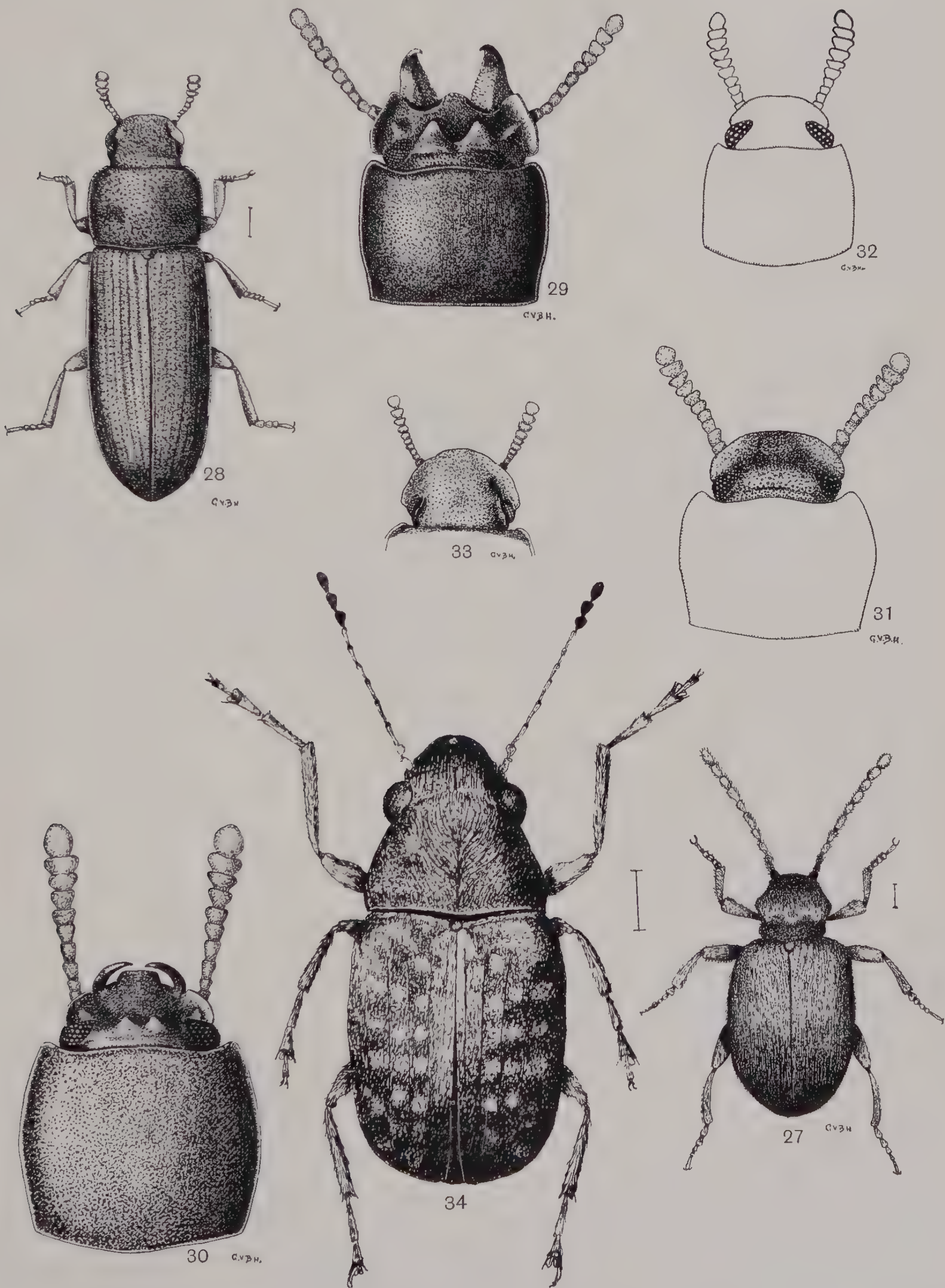

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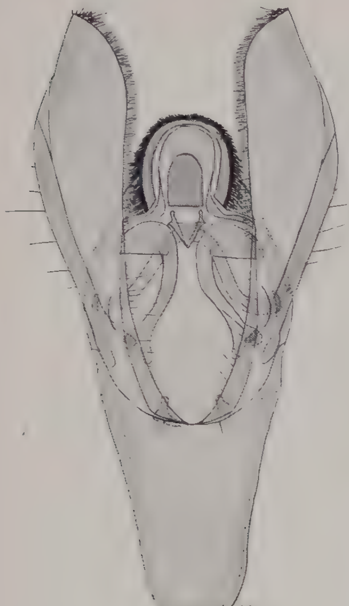

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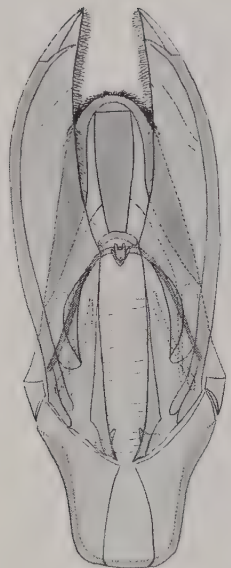

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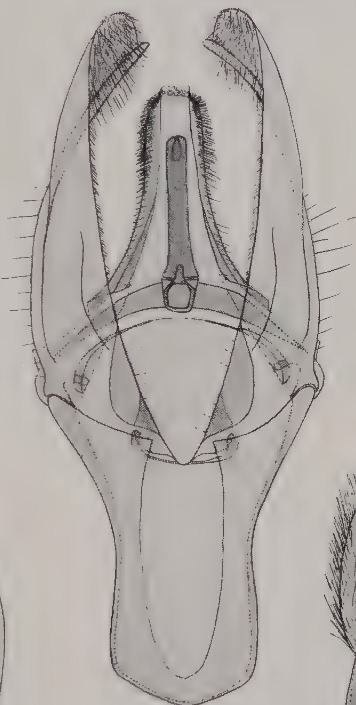

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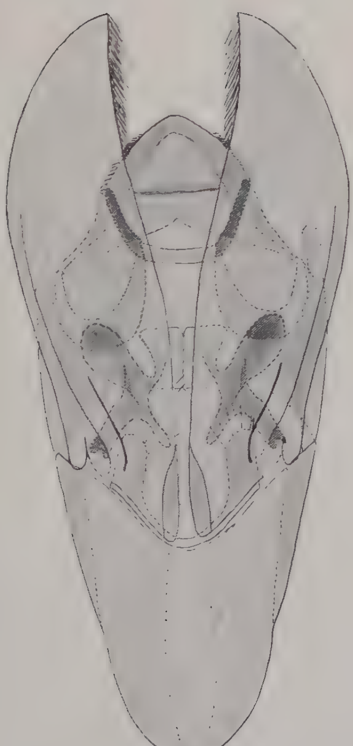

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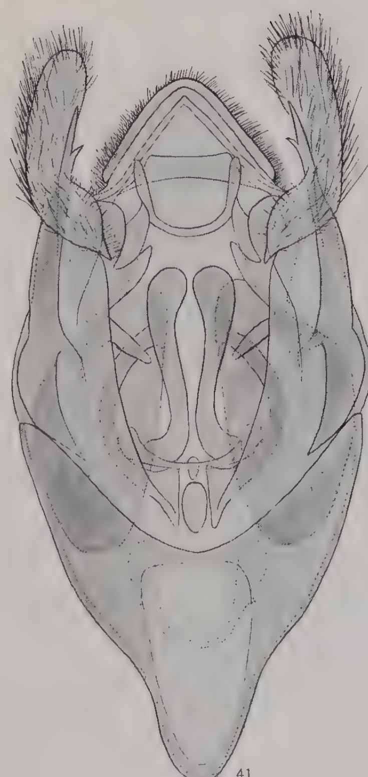

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RICHARDS & HERFORD.—INSECTS FOUND WITH CACAO, ETC., IN LONDON WAREHOUSES (pp. 367–395).


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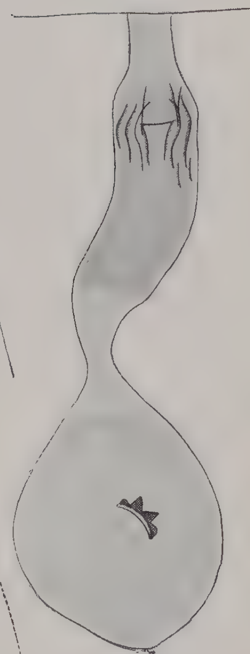
43b


46a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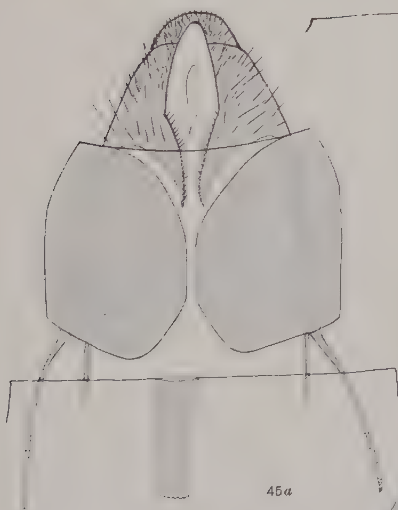
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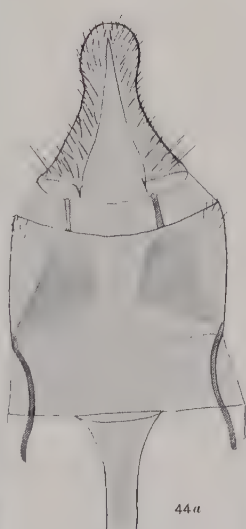
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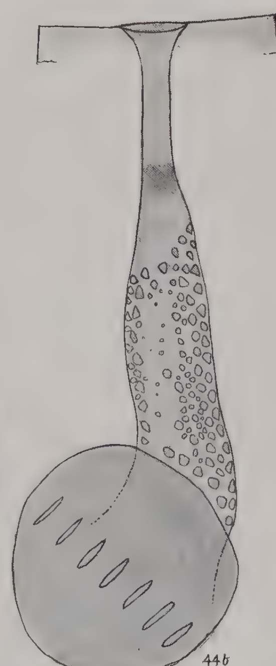
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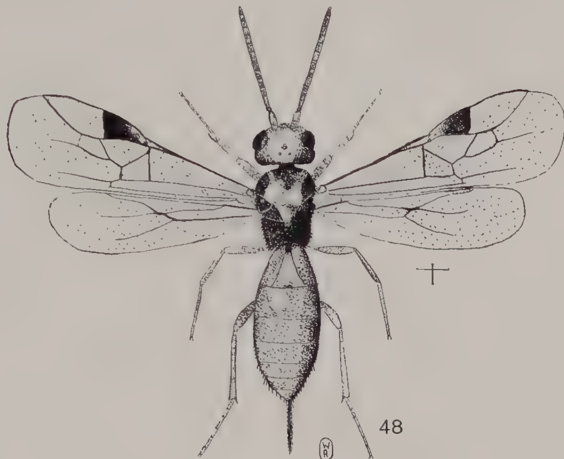
RICHARDS & HERFORD.—Insects found with Cacao, etc., in London Warehouses (pp. 367–395).



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RICHARDS & HERFORD.—INSECTS FOUND WITH CACAO, ETC., IN LONDON WAREHOUSES (pp. 367–395).

PLATE XXVI.

Fig. 27. *Ptinus tectus* Boield.
Fig. 28. *Tribolium castaneum* Herbst.
Fig. 29. Head and thorax of *Gnathocerus cornutus* F., male.
Fig. 30. The same of *G. maxillosus* F., male.
Fig. 31. The same of *G. cornutus*, female.
Fig. 32. The same of *G. maxillosus* F., female.
Fig. 33. The head of *Tribolium confusum* Duv.
Fig. 34. *Araecerus fasciculatus* De G.

PLATE XXVII.

Fig. 35. *Plodia interpunctella* Hb.

PLATE XXVIII.

Fig. 36. *Ephestia elutella* Hb.

PLATE XXIX.

Fig. 37. *Corcyra cephalonica* Staint., male.

PLATE XXX.

Fig. 38. Male genitalia of *Ephestia elutella* Hb., ventral view, after removal of the oedeagus.
Fig. 39. The same of *Ephestia kühniella* Zell.
Fig. 40. The same of *Ephestia cautella* Wlk.
Fig. 41. The same of male *Ephestia* from Cameroons cacao.
Fig. 42. The same of male *Plodia interpunctella* Hb.

PLATE XXXI.

Fig. 43 (*a*) Ventral view of the female genitalia of *Ephestia kühniella* Zell.
(*b*) The bursa copulatrix of *E. kühniella*, female.
Fig. 44 (*a*) Ventral view of the female genitalia of *Ephestia elutella* Hb.
(*b*) The bursa copulatrix of *E. elutella*, female.
Fig. 45 (*a*) Ventral view of the female genitalia of *Ephestia cautella* Wlk.
(*b*) The bursa copulatrix of *E. cautella*, female.
Fig. 46 (*a*) Ventral view of female genitalia of *Plodia interpunctella* Hb.
(*b*) The bursa copulatrix *P. interpunctella*, female.

PLATE XXXII.

Fig. 47. *Holepyris hawaiiensis* Ashm., female.
Fig. 48. *Microbracon hebetor* Say, female.

(*Received July 2nd*, 1929.)

REVIEWS

The Plant Rusts (*Uredinales*). By Joseph C. Arthur. Pp. iv + 446, with 186 figures. London: Chapman and Hall, Ltd. 1929.

During the first thirteen years of the century a number of workers published books on the rust fungi: Fischer in Switzerland, Klebahn in Germany, Hariot in France, McAlpine in Australia and Grove in England. Since then no volume has appeared on this group although a great amount of research has been carried out. One of the most prolific centres of rust research has been the laboratory of Prof. J. C. Arthur of the Agricultural Experiment Station in Indiana, and he and a group of his old students and colleagues (Kern, Orton, Fromme, Jackson, Mains and Bisby) have now brought together much of their work in this timely volume. A great part of this research has been taxonomic and the results have appeared in Volume VII of the great *North American Flora*. The more general and biological aspects of these studies have been presented from time to time, in a fragmentary way in sporadic papers, but the need for their more adequate expression was evident. This need is met by the present volume which is a mine of information.

Each author's contribution has apparently had to pass the criticism of a majority of the symposiasts but there is no individual responsibility, the contributions having been dovetailed together by Prof. Arthur. Such a method has produced a "safe" readable book, but one cannot help feeling that it has also pruned out a good deal of originality and spontaneity. In spite of this the book is a fine piece of work redounding to the credit of the authors and their leader.

The first two chapters are introductory. Chapter I deals with the general nature of the rust fungi; their reproductive structures and developmental cycles. On the second page there is the basis of a pretty controversy in the adoption by the authors of the concepts and terms *gametophyte* and *sporophyte*. Chapter II is a historical survey of the rust fungi, beginning with Old Testament records, which one is glad to see treated with commendable scepticism, and closing with a discussion of modern trends of research.

Chapter III, which begins the more detailed treatment, is devoted to a consideration of the ontogeny and phylogeny of the rusts and the bases of their classification. The contents are a fair and balanced treatment of a number of controversial questions. So much of the attention of mycologists has been focussed on the relatively easy and obvious problems of reproduction and spore forms, that one is glad to see the authors taking the point of view that "the rusts have a vegetative body that holds such a prominent place in their development that it requires serious consideration." This is true of most fungi and, when this more obscure avenue is explored, our understanding of the fungi will make considerable advance: it really is astonishing how little we know about fungus mycelium in its actively functioning vegetative condition. Speaking of heterothallism, the authors remark: "The subject suggests vistas of valuable information." This is mild appreciation: heterothallism, with all its implications, is one of the most important and far-reaching avenues of study open to mycologists to-day. The chapter contains a fair summary of current views on rust phylogeny including Mez and Ziegenspeck's serodiagnostic studies but, frankly, after reading these pages one is left wondering whether any of these speculations have the slightest validity; in fact, whether speculation of this kind is, in any way, worth while at present.

Chapter IV is the more usual type of approach to the study of the fungi; dealing with morphology and cytology, but still being a useful account which incorporates much recent work. Chapter V contains an interesting and suggestive account of the dissemination and geographic distribution of the rust fungi. Both of these are aspects

of study which have in the past been curiously neglected, and in many considerations of the fungi have been entirely omitted. It is a little difficult to understand why this should be so, for both aspects are obviously not only economically important but scientifically profitable lines of research.

Chapter VI is again a more usual method of approach to fungus study, and contains a brief but adequate general account of our knowledge of the physiology of the rusts, attention being paid both to physico-chemical and biological relationships. One is glad to see attention drawn to a point of view for which mycologists in general seem to have a remarkably blind spot, viz. that the development of a parasite need not be and, in the rust fungi, usually is not in inverse ratio to the vigour of the host. The idea of the inverse ratio of host and parasite with a see-saw balance is a direct anthropomorphic projection and is a generalisation which, in plant disease, entirely lacks validity.

Chapter VII, on specialisation, deals with one of the most interesting fields of rust research, of fundamental importance both economically and scientifically, and an aspect of rust study on which an enormous amount of literature has accumulated. Curiously enough, this chapter is one of the shortest in the book, which is a pity, for it might with great advantage have been made much fuller and written in a less condensed and more readable style. As it stands, however, it is one of the most important chapters in the book, especially in view of the resuscitation in Europe—where indeed it had never been abandoned—of the bridging host theory. The end portion of this chapter dealing with stability of reaction is, in view of its primary importance, so brief as to be almost exasperating: a fuller consideration would not have upset the balance of treatment in the book.

Chapter VIII deals with abnormalities and diseases firstly, of the fungi themselves and, secondly, of the host plants. Fungus teratology is an interesting field of which little is known but which obviously presents a fine avenue for genetic exploration and for the experimental study of morphology. The consideration of abnormalities produced in hosts by rust attack is brief but adequate. There are few fields of plant pathology presenting more interesting problems in morbid anatomy than the host reaction in rust diseases, and it is to be hoped that this suggestive discussion will draw attention to these problems which have been much neglected outside of Germany.

Chapter IX is a brief survey of the more general aspects of the rust diseases of the several types of crops. It, wisely, does not attempt to compete with an ordinary text-book of plant pathology, but is rather of the nature of a running commentary to such a text-book.

The last chapter is a useful account of methods of investigation. It does not seem to be written for quite the same class of reader as the rest of the volume and, here and there, is distinctly naïve—*e.g.* "some knowledge of hosts is required of one who would be a really successful collector of rusts." The treatment of spore measurement is brief and inadequate: surely it is now sufficiently recognised that range of spore size is uncertain and may be misleading, whilst 10 to 12 individual spore measurements are insufficient as any critical basis.

The book closes with a useful bibliography which is recognised to be far from complete, and a good index. Additional fungus and host indices would greatly have facilitated the use of the volume. A notable feature are the illustrations, many of which are unusually well reproduced, although Figs. 106, 174 and 182 might have been omitted without any loss.

The book is essentially not a monograph but is a wide and knowledgeable survey of the general biology of the rust fungi. The absence of a more detailed consideration of particular problems is at times almost exasperating, and every reader will have views about sins of commission and omission and the relative space allotted to the several topics. One has to recognise, however, that the volume, as it stands, is a brilliant and well-balanced production. There is no question of the importance of the book and it is quite indispensable to all botanical and pathological laboratories. In the former it should have a most valuable influence in extending interest in the rust fungi among students, and in giving a wider and more biological foundation to the

teaching. It is to be hoped that workers on other fungi will come together as these authors have done and produce parallel volumes—a companion volume on the smut fungi is obviously indicated.

WILLIAM B. BRIERLEY.

Plant Ecology. By JOHN E. WEAVER and FREDERICK E. CLEMENTS. 8vo. Pp. xx + 520. Figs. 262; coloured map. New York: McGraw. Hill Book Co. 1929. 25*s*. net.

The science of Ecology is rapidly growing out of its youthful stages and is showing many signs of maturity. It has had a prolonged adolescent and youthful period, during which it has energised in all directions, accumulating data, crystallising out its concepts, formulating principles and trying to coin and apply a satisfactory terminology. Neither principles nor terminology have as yet met with complete acceptance, but during the last few years there has been a notable trend in this direction. Ecology, rather more than other aspects of botany, has developed distinct schools and one of the most active of these is associated with the name of Clements. The dynamics of vegetation as expressed in plant succession has been the main theme of this school, and during nearly 30 years' volumes, which are landmarks in the massing of ecological data and the formulation of ecological theory, have issued in a kind of magistral procession. The present volume, some 500 pages, deriving from Clements and one of his old students, Weaver, crystallises out in text-book form these three decades of work. The collaboration is peculiarly happy for, speaking very broadly, Clements has been a shoot investigator and Weaver a student of roots.

The authors state that: "The volume is designed to meet the need for a comprehensive text-book of plant ecology and to furnish a guide to workers in related fields. It is written from the standpoint of development, instrumentation, and experiment. The student of plant production, whether in botany, agriculture, grazing, forestry, plant pathology, or other fields, is beginning to study more thoroughly the intimate relations between plants or groups of plants and their environment. In fact, many of his most important problems deal with the relations of plant to habitat, whether the latter be natural or modified by cultivation, and these cannot be satisfactorily solved until these relationships are well understood. In addition, the field of ecology is unique in its fundamental contributions to a general understanding of the plant world upon which man and animals are dependent. This book has been planned to meet these several needs." The book is a great advance on previous works in condensation and comprehensiveness and gives one a feeling, that is often absent when reading ecological works, of solidity and scientific accuracy. It contains a wealth of information which, in less skilled hands, would have remained an incoherent and indigestible mass but which, here, is marshalled in logical order and discussed in a broad and convincing manner.

The chapter contents, which are as follows, show the wide scope of the volume: (I) Vegetation; its origin, development and structure; (II) Methods of studying vegetation; (III) The units of vegetation; (IV) Plant succession; (V) Initial causes of succession; (VI) Aggregation, migration and ecesis; (VII) Competition and invasion; (VIII) Reaction and stabilisation; (IX) Factors of the habitat; (X) Relation of underground plant parts to environment; (XI) Humidity, wind and evaporation; (XII) Temperature; (XIII) Light; (XIV) Plant response as a measure of environment; (XV) Adaptation to water; (XVI) Relations between plants and animals; (XVII) Plants and plant communities as indicators; (XVIII) Climax formations of North America.

The chapters are distinctly unequal, although probably each reader will have his own views as to their relative value. To this reader it would seem that Chapters III, IX and XVI are below the standard set, and XVI particularly might well be re-written in the next edition of the book. Parts of Chapter XV already need considerable modification in view of Maximov's work. On the other hand, Chapters IV, VII and X are splendid expositions of these problems.

Prof. Clements is known to hold views regarding terminology and to be unusually prolific in the coining of new terms. One opened the volume, therefore, with apprehension as to its qualities of readableness and clarity. Very wisely, the authors have restrained their pens except perhaps, as was almost inevitable, when dealing with units of vegetation, but against this it may be said that most of these terms have proved their value and are now established. Here and there throughout the book one meets unexpected words such as "photosynthate," "forb," etc., but these are few in number and always explained. Occasionally the technical terms tend to make sentences rather ugly, *e.g.* "Most of the migrules are carried to areas already so populated that the newcomer can not ecize." Americanisms are not infrequent, *e.g.* "The lichens help corrode and decompose the rock," or "By fall, 25 per cent. of the grass had died," but as the authors are American one cannot grumble at this, and one must be glad that, on the whole, the book is written in such very clear and simple English.

An unsatisfactory aspect of the book is the absence of uniformity in dealing with measurements. The authors play rather fast and loose with English and metric systems, and temperatures are given indiscriminately as Fahrenheit or Centigrade; occasionally one must guess from the context which scale is meant. Frequently metric and English measures or Fahrenheit and Centigrade scales are both used in the one paragraph or tabulation and occasionally even in the one sentence. This attitude shows an open mind but is irritating for the reader.

Interspersed through the text are small type directions for practical study. Of these the experiments and exercises for greenhouse and laboratory work are outlined in detail, while suggestions for field work are only given in broad outline. These directions together with a special note at the beginning of the volume will prove of value in guiding the teacher. There are also some 262 well-chosen line and photographic illustrations.

Terminating the volume is a good index and a bibliography. The latter illustrates an unsatisfactory feature of the book—it is too American. In spite of the authors' statement that the "volume is designed to meet the need for a comprehensive text-book of Ecology," the work is frankly an American text-book designed for American students. As a matter of fact, the discussions all lead up to the final chapter on Climax Formations which is really the crown of the work—and which deals solely with American formations. Even so, in a volume of this size and quality, one would have expected to find some reference to the work of Braun-Blanquet, Brockmann, Diels, Domin, Erdtman, Keller, Lindquist, Lundegardh, Markgraf, Oliver, Pavillard, von Post, Rietz, Rudolf, Skottsberg, Stoyanoff, Szafer, Uehlinger, Wulff and numerous other well-known European students. Of the 606 references in the bibliography only about 120 derive from outside the United States; which is really too small a proportion.

These criticisms deal with matters which, although of subsidiary importance, tend to mar what is the most serious contribution of its kind since Warming's *Oecology of Plants*, and a work which is bound to have an immense influence on the teaching and development of the subject.

An outstanding and most welcome feature of the volume is the culling of data from agriculture and forestry as well as the untutored vegetation of the globe, and the application of ecological principles to farm and forest crops. The recent Scandinavian and Russian developments have demonstrated how intimate is this relation of Ecology to agriculture, and our own Empire studies show the same for forestry. The relation cannot be over appreciated, for agriculture and forestry are two of the great source books of ecological research and much of their practice is simply applied ecology. This is more clearly recognised in agricultural America than in industrial Europe and its clear exposition in the present volume is noteworthy.

Botanists of all kinds will find this volume interesting and useful, and will accord very grateful thanks to Prof. Weaver and Prof. Clements for the fine work they have produced.

WILLIAM B. BRIERLEY.

PROCEEDINGS OF THE ASSOCIATION OF ECONOMIC BIOLOGISTS. I

Ordinary Meeting held at 2.30 p.m on Friday, November 22nd, 1929, in the Botanical Lecture Theatre of the Imperial College of Science and Technology, London. The Chair was taken by Dr J. Waterston, Vice-President.

RESEARCH ON INFESTATION OF STORED PRODUCTS.

I. Entomological Aspects:
- (*a*) Survey and Inspection Work. By W. S. Thomson.
- (*b*) Biological Work. By G. V. B. Herford.

II. Mycological Aspects. By R. H. Bunting.

I (*a*). ENTOMOLOGICAL ASPECTS—SURVEY AND INSPECTION WORK.

By W. S. Thomson.

The problem of insect infestation has three main aspects: infestation in the exporting country; in the wharves and warehouses in the importing country; infestation in the cocoa and chocolate factories.

During the last two years, survey work has been concerned principally with the second of these aspects, and has been designed to reveal if possible which insects are chiefly responsible for infestation of cacao; the extent to which infestation takes place at different seasons and in cacao from different countries; to define the extent to which re-infestation occurs in the warehouses in this country, and to ascertain the extent to which infestation is preventable.

From the mass of data collected during this time the following points of interest and importance have emerged.

While about thirty species of insects have been found associated with cacao, only four are of economic importance: these are the Phycitid moths, *Ephestia elutella* Hb. and *E. cautella* Walk., the Galleriid moth, *Corcyra cephalonica* St., and the Anthribid beetle, *Araecerus fasciculatus* De G. Taking cacao as a whole, the two Ephestias are the most important of these.

There is considerable infestation by insects of cacao on arrival in this country. No cacao is immune, but that from some countries seems less liable to severe infestation than that from others. Infested cacao arrived during each month of the survey, both summer and winter, but there was a period of maximum infestation which extended from July to October.

Araecerus fasciculatus, while frequently found in West African cacao, is absent from most other cacaos. It occurs abundantly in Grenada nutmegs, but although

these are frequently shipped home with Granada cacao, the cacao seems almost free from its attacks.

The Braconid, *Microbracon hebetor* Say, parasitises the larvae of *Ephestia*, often very heavily—especially in September and October, but is probably of little use for purposes of biological control.

It is almost impossible at this stage to assess the loss caused by insect infestation of cacao. Losses are due to destruction of cacao itself by insects, to fouling of other products, *e.g.* arrowroot, by larvae of *Ephestia* crawling over it from cacao and leaving their web behind them, and to loss of prestige of manufacturers by infestation of the finished product.

Remedial and control measures to be effective must begin in the exporting country. In this country only palliative measures can be adopted at present.

In most of the warehouses under survey other products are stored adjacent to cacao, *i.e.* spices and coffee. The fauna of a cacao and spice warehouse can be divided broadly into five classes: primary pests; secondary pests; predators, general scavengers and mould feeders; species accidentally introduced and not having any real connection with stored products; and parasites.

I (*b*). ENTOMOLOGICAL ASPECTS—BIOLOGICAL WORK.

BY G. V. B. HERFORD.

THE biological work in connection with the research into insect pests of stored products has not been in progress so long as the survey has, the main problems had to be discovered by the latter branch before any laboratory work could be attempted.

There are two main lines of approach in this work, and they both have a common goal, the final control of the insect pests concerned.

One necessitates the use of fumigants and other means of chemical and physical control, such as heat or cold. In certain cases it may be necessary to employ these methods, but it is felt that they should be considered as palliative rather than final, as a cure as opposed to a prevention.

At the laboratory at Slough a fumigation plant and a refrigerating plant are being installed, and this side of the work will receive full attention.

Turning to the more purely biological aspect, attention is being directed upon a close study of the ecology of certain insects, notably *Ephestia elutella* Hb., the Cacao moth, and *Plodia interpunctella* Hb., the Indian meal moth, the most important pest of dried fruit. It is hoped that by studying the effect of different degrees of humidity and temperature upon the life history of the insects concerned, a means of control may be discovered, at once effective, simple and final.

These long range experiments are of necessity of long duration, and this fact makes clear the need for work upon the palliative measures mentioned above.

For all these experiments, and particularly those of fumigation or freezing, very large stocks of insects have to be reared. As an instance of this it may be mentioned that in one of a series of freezing experiments that were being undertaken in co-operation with a large firm of caterers between 2000 and 3000 eggs of *Plodia* were used. To be able to obtain this number of eggs at any given time it is necessary to have a very large stock, and the successful rearing of such stocks has been and will be for some time to come one of the most difficult parts of the work.

Many types of breeding cages have been tried with varying success, ranging in size from special boxes under a foot long for small stock, to dustbins, which were used in an endeavour to breed *Plodia* on a very large scale. The cages in use at present have been designed for their especial purpose, and appear to be giving satisfaction.

Two large insectaries are being built, in which warehouse conditions are to be imitated as far as seems desirable, and in these suitable pests and their foods will be placed, in the hope of obtaining a really bad infestation. Besides being a means of raising large stocks this may provide valuable evidence of the causes of infestation as occurring in the docks.

There are many factors to be contended with in this building up of large stocks of insects. Temperature and humidity play a most important part in their effect on the insects themselves and also upon the foodstuffs. Moulds, favoured by excessive humidity, will soon destroy a flourishing culture, while hymenopterous parasites, mites and bacteria, which are not usually effective as a control in the warehouses, are a most potent source of destruction in the crowded conditions of a breeding cage or insectary. For their elimination a stringent quarantine service has to operate upon all insects brought to the laboratory from the docks before they can be added to healthy cultures.

For experimental work upon life histories under varying conditions a constant temperature and humidity room is being fitted up in the laboratory. As it is not yet completed, it is impossible to give any figures for the accuracy of control which will be finally attained, but the results so far are quite promising.

It must be borne in mind that this work is still in its infancy, and consequently this paper is an account not so much of work accomplished as rather an indication of the main lines to be followed. Already very many side problems have arisen, and will appear more and more as the work progresses. In connection with fumigation there arises the whole problem of the respiration of insects, about which all too little is known, while a physiologist and chemist could find almost unlimited scope for research in the relations between the insect and its foods.

II. MYCOLOGICAL ASPECTS.

By R. H. Bunting.

The mycological work of the Stored Products Research Station has up to the present been centred on problems connected with the deterioration of cocoa beans by mould infection. Since this work forms a natural corollary to work done, and in progress, in the Gold Coast Colony, it may be well briefly to review investigations made in that country.

The Gold Coast furnishes by native production nearly half the world's demand for raw cacao. This produce, because it is obtained from an inferior variety of *Theobroma cacao*, and because the standard of its preparation is not always high or uniform, does not realise so good a price as does cocoa from other parts of the globe. It is consequently used, and has become indispensable as a foundation to which better beans are added for the sake of quality in the manufacture of chocolate and cocoa powder. There is always a big demand for this cheaper grade of beans, and considerable competition is displayed by buyers in immediate contact with the native

producer in the indiscriminate purchase of beans which may or may not be sufficiently dried or well prepared. Cocoa farmers, though frequently illiterate, are by no means unintelligent, and if they can sell water at the local price of cocoa, they do not take unnecessary trouble to dry their produce thoroughly—an operation sometimes difficult because of climatic conditions. It may, therefore, be well understood that far too high a percentage of beans prepared and sold in the humid conditions of the Gold Coast rain-forest, become internally mouldy before the European merchant receives them at the larger buying centres for export. He does what he can to prevent further deterioration by exposing the produce to sun-heat, but the fact remains that mouldy beans do reach the European and American markets.

Manufacturers of cocoa and chocolate dislike beans affected by internal moulds because of the objectionable flavour they impart to their products, the increased acidity of the cocoa butter, and a disintegration of the cotyledons caused by moulds. Whether there is any justification for refusing acceptance of mouldy beans on account of the pathogenicity of the organisms concerned is not known, but the pure food legislation of the United States of America refuses entry to consignments containing more than a small proportion of moulds, and mouldy cocoa is frequently the cause of arbitration and financial loss in other importing markets.

No distinction is made in the cocoa trade between the species of fungi found in the produce—all are "moulds"—a term which has been known to include chemical incrustations caused by contact with concrete. But in our work on the Gold Coast it became apparent at the start that it would be necessary to differentiate the species involved as accurately as local facilities permitted, because of the different conditions under which they occurred. It was found convenient to divide the moulds into three ecological groups: Thermophiles, which occurred with some frequency in fermenting masses of beans; Hygrophiles, found in beans with an excessive moisture content, and those forms which occur in beans considered to be commercially dry.

As an example of the complexity of problems relating to the moulding of cocoa beans, it may be mentioned that in several instances where indeterminate mycelia were found infecting embryos, one-half of a bean incubated, under aseptic conditions, at 40° C. resulted in one or other of the thermophilic moulds, whereas the complementary portion of the bean grown at laboratory temperature developed one or more of the other moulds. The commoner of the moulds were confined to the orders Mucoraceae and Aspergilleae. Other fungi were occasionally found, such as *Botryediplodia theobromae* in beans inadequately prepared from diseased pods, *Cladosporium* sp. on beans stored for a long time, *Monilia sitophila* was of still more rare occurrence, whilst many searches for Actinomyces, with one possible exception, gave negative results.

The genus *Aspergillus* provides the chief offenders in Gold Coast cocoa beans, its species including about ten members of the following groups and species—*A. fumigatus*, *A. flavus*, *A. glaucus*, *A. ochraceus*, *A. tamari*, *A. niger* and probably *A. sydowi.* Of the Mucoraceae, a new thermophilic species of *Mucor*, *Absidia capillata* and *A. regneri* and *Circinella spinosa* have fairly commonly been found.

Having obtained some information regarding the forms occurring in cocoa beans and the conditions under which they occurred, it was obviously desirable to ascertain their method of entry, since it is their presence on the embryo of the seed that is objectionable to manufacturers. A very large proportion of the many thousands of

mouldy beans examined gave distinct evidence of entry having been made at the micropylar end of the beans, and Dade confirmed the evidence by microtome sections which demonstrated hyphae penetrating the testa at that point. The point of penetration is interesting. The radicle of a germinating cocoa seed does not cause an irregular rupture of the testa, but its emergence is provided for by the removal of a disc of the tissue which leaves a characteristic, clean cut, circular hole. Microscopic examination of the tissue of this region shows no apparent structural differences, and probably the removal of the plug is due to enzymatic action. The early stages of the fermentation processes provide conditions which encourage germination, so that it appears probable that the entrance of the fungal organism at the micropylar end of the testa is assisted by an incipient germination set up during the early stages of fermentation. This view is confirmed by the long noted fact that fermented beans are much more frequently affected by internal moulds than are unfermented beans. Dade showed that *Aspergillus fumigatus*, the member of the *glaucus* group, which we consider to be *A. chevalieri*, and possibly the thermophilic *Mucor*, are capable of penetrating the testa of fermented beans at the micropylar region.

In the Gold Coast these three species may be considered as the most economically important of the moulds affecting cocoa beans, with possibly the addition of *A. sydowi*. *A. fumigatus* and the *Mucor* occur in fermenting heaps and though they are apparently slower to develop within commercial cocoa beans than other species, their entrance permits that of the latter. *A. fumigatus* moreover belongs to a specific group, members of which are known to be human pathogens.

The species, *chevalieri* and possibly *sydowi*, are of considerable—possibly greater—importance since their low moisture requirements permit commercial transactions in beans not obviously subject to deterioration by too high a moisture content.

By a series of careful experiments to determine the degree of dryness necessary to prevent moulding, the percentage of water content which permitted the development of *A. chevalieri* was found to lie between 8 and 9·5. As the other moulds were shown to require a higher water content, these figures were considered to be critical, and a practical method for testing whether samples contained more or less than 7·8 per cent. of moisture was evolved for the use of cocoa traders.

During the course of this work it had been found that internal infection might take place during the process of fermentation in a large proportion of the mass under native treatment. It was also evident that the common practice of drying the fermented beans on trays in the open air continued the exposure to infection. Unwashed beans, covered and saturated with the remains of the mucilaginous contents of the pod, provide an excellent medium for the growth of moulds. So that the conclusions we arrived at, that any beans which remained, or became sufficiently damp might be expected to become mouldy, and that all beans prepared by native methods were potentially mouldy, seemed justified. It was desirable, therefore, to ascertain the reaction of prepared beans to climatic conditions. Without going into the details of a number of experiments designed for this purpose, it will be sufficient to state that by laboratory methods it was found that cocoa beans are extremely hygroscopic, and that in four days they come to within 1 per cent. of equilibrium with a stable atmosphere, either by absorption or dehydration, the critical water contents 8 and 9·5 per cent. being at equilibrium with atmospheres of 82 and 87 R.H. respectively. Climatic conditions are, however, never stable in the Gold Coast, and cocoa beans

are very sensitive to daily fluctuations in R.H., the high humidity of the night hours increasing the moisture content of the beans, and the drier atmosphere of daytime decreasing it with rapid reaction. Whilst these daily fluctuations prevented the establishment of a perfect equilibrium between moisture content and atmospheric moisture, it was found that the average of readings taken at 4-hourly intervals gave a guide to the moisture content which was sufficiently accurate to be useful for practical purposes. In practice this information indicated that in certain places cocoa could not be stored without risk of mould, unless some protection against atmospheric moisture were provided. And it made apparent the fact that good storage conditions were just as important a factor in the prevention of moulding as was proper drying. Incidentally, the blame for decreasing the value of the produce by permitting moulding can thus be distributed between merchant and producer to the possible relief of the latter.

In the work related above, cocoa beans had been dealt with as hygroscopic entities, but it was known that a great difference existed between the hygroscopic properties of testa and embryo. A number of somewhat difficult tests showed that the seed coat was far more active in its reaction to external moisture conditions than was the embryo. So that beans having the same total moisture content might show considerable differences between the water in their testae, with proportionate differences in their embryos, until the testa came into equilibrium with the embryo and with the atmosphere. This fact is of some practical importance from the view-point of infection, since internal infection, depending as it does upon preliminary external infection, requires a sufficient amount of water in the testa for the initial development of the fungus, and beans during the drying process which succeeds fermentation, *i.e.* whilst rapidly losing moisture from the testa—are much less liable to infection than are equally moist beans which are absorbing moisture. Slow and protracted drying also permits internal infection owing to the fact that the testa does not pass into the atmosphere moisture absorbed from the embryo at a sufficiently rapid rate.

There remains much to be done on the Gold Coast on questions relating to moisture and moulds in cocoa beans, and the work already accomplished is intended to form the basis for investigations of a wider and more commercially useful nature. In such investigations the staff of the Stored Products Research Station hopes to assist. It is obvious that the first necessity in such co-operation is to determine the identity of the organisms causing deterioration of cocoa beans when landed in this country, in order to compare them with those found in the country of origin. And since the condition of the produce is of more importance to the manufacturer than to others, it will be necessary to examine cocoa beans after varying periods of storage. Identification of cocoa moulds is not so simple a matter as one could desire when inspecting material in quantity, but the degree of accuracy in delimiting the strains and mutations of a species need not be particularly high for other than a few of the more important moulds selected for critical study. What these species are likely to be depends upon the incidence of their occurrence, and the conditions under which they are found, but it is probable that *Aspergillus chevalieri* and *sydowi* are worthy of detailed study. A large number of references can be found in mycological literature to work done on members of specific groups of such genera as *Aspergillus*, etc., but sufficient information is rarely given to satisfy one that it relates to any particular

strain. It is therefore possible that a good deal of time may be occupied on work which may later prove to have been done before.

The Slough Station is fortunate in having obtained a promise of co-operation in the analysis of flavour effects produced by cocoa moulds, from Mr Macara of the British Research Association of the Cocoa, Chocolate and Allied Trades. Such assistance will provide more time being spent on what is likely to become the routine work of examining material received from the docks, and will permit greater attention being paid to systematic and observational work of fundamental importance.

By the courtesy of a number of importing firms, facilities have been granted us for the examination of consignments of cocoa coming into London and Liverpool. Samples of these consignments will be taken in air-tight tins, their moisture-content ascertained and their fungus flora analysed. By this means it is hoped to gain some idea of the comparative deterioration by moulds in cocoa from all parts of the world, and an indication of the moisture requirements of these moulds. Special advantage will obviously be derived from any consignment which has been examined by produce inspectors before transport by sea.

This more or less casual collection of material, depending as it does upon the goodwill of certain importing firms, is not expected to provide more than an incomplete story of the produce entering this country. It may not permit the examination of the lowest grade of beans which no one is proud to own, and which might be expected to yield interesting economic and possibly mycological data.

With regard to this class of cocoa beans, one ought to have made previous mention of the fact that in addition to insufficiently dried beans and those which have absorbed atmospheric moisture after having been dried, another cause of internal moulding is direct contact with either fresh or rain-water. Such cocoa is recognised by insurance agents as "rain- or sea-damaged," and does not require investigation, the cause being obvious, and the solid mass of mycelial growth which masks the original contents of the bag yielding but little intelligible information.

For detailed observations on beans of known moisture content and fungoid infection, arrangements have been made for consignments to be sent under ordinary transport conditions from the Gold Coast. A series of such trial consignments, duplicated in moisture-proof containers as controls, may reveal the effects of storage in a steamer's hold sufficiently well to indicate the desirability of more accurate, and extended, observations being made on the various climatic conditions which obtain in different parts of a cargo of bagged cocoa. It is conceivable that, during transport by sea, the draught of a ventilating shaft may actually lower the number of commercially mouldy beans in bags stowed in its path, and that beans in the proximity of a bulkhead on which moisture is condensing, or of wet bags, may become commercially moulded.

It is intended to utilise the bags of cocoa derived from the Gold Coast for periodic observations under normal storage conditions in riverside warehouses, and at the same time to record the climatic conditions which obtain in those stores. By comparison of the humidity and temperature of the centre of a stack of bagged cocoa, with those of the general atmosphere of the store, it is hoped to obtain data which may be useful to cocoa merchants. The conditions controlling mouldiness within the protective walls of a cocoa sack are being investigated in the more humid environment of the Gold Coast.

Before leaving that Colony, work was started on the moulds affecting copra there, and they were found to be apparently the same organisms as those infecting cocoa beans. Since it is probable that copra moulds are much the same whatever the country of origin, it has been decided to run copra mould investigations at Slough concurrently with cocoa moulds. The problems are, however, not very similar. In cocoa it is, broadly speaking, the presence rather than the action of the fungus which is objectionable; in copra the presence of some moulds is thought to be an advantage by certain manufacturers, because of their beneficial action in destroying cellulose, or of protecting the surface of the copra from other moulds, whilst the action of many moulds is more or less disastrous on this produce.

It is probable that the questions connected with copra moulds in this country may necessitate a greater amount of fundamental work than do those questions which relate to cocoa moulds, and as a start Mr Eyre is studying the enzymatic action of *Aspergillus chevalieri* on copra. Very little literature is available which deals with the reaction of copra to atmospheric moisture, and with the effects of preparation on moulding, but the recent appointment of a specialist officer to the Malayan Department of Agriculture for the purpose of elucidating such problems is decidedly hopeful. Needless to say, the Stored Products Research Station has opened up lines of communication with him.

In conclusion, may one add that the staff at Slough desires to render any mycological assistance within its power to those interested in the deterioration of stored products by moulds?

PROCEEDINGS OF THE ASSOCIATION OF ECONOMIC BIOLOGISTS. II

Ordinary Meeting held at 2.30 p.m. on Friday, December 13th, 1929, in the Botanical Lecture Theatre of the Imperial College of Science and Technology, London. The Chair was taken by the President, Dr E. J. Butler, C.I.E., F.R.S.

APPLE SCAB, ITS INCIDENCE AND CONTROL.

Prof. E. S. Salmon, South Eastern Agricultural College, Wye, Kent.
Mr F. R. Petherbridge, School of Agriculture, Cambridge.
Mr M. H. Moore, East Malling Research Station, Kent.

I. Prof. E. S. Salmon.

Prof. Salmon said: The subject of Apple Scab can be treated from many different angles; in the time at my disposal I should like to consider the control of apple scab in its practical aspect as it has to be faced on the farm.

The control of apple scab is not a single problem, but rather a number of problems. We are all rather inclined to rush out and show how the disease can be controlled by spraying, and then we discover very often what a number of problems there are to be solved before we can say to the farmer: "Carry out this programme and you will grow clean apples." Let me enumerate some of the chief problems.

The life history of the fungus. Let us consider the life history of the fungus in relation to the primary infection of the apple tree in spring. In this country the fungus may persist from season to season both in the conidial (*Fusicladium*) stage on the one-year-old wood and in the perithecial (*Venturia*) stage on dead scabbed leaves.

Much labour and money have been unnecessarily expended in the past—and the practice still prevails in some parts of the country—on spraying in the winter in the hope of killing the fungus on "scabbed" wood. Apart from the fact that the scab pustule may remain covered by the bark until spring, it is very doubtful if spraying can kill the mycelial cushion of the scab pustule. Although in some cases scab pustules on the wood may be responsible for the primary infection of the leaves in the spring, this source must, I think, be considered negligible compared with that afforded by perithecia ejecting ascospores from the dead scabbed leaves on the ground. Frequently, moreover, in orchards and plantations where the disease is severe, scabbed wood is absent or present in only a very small amount.

Much investigational work concerning the perithecial stage of apple scab has been carried out, particularly in recent years. We know from the work of Aderhold on the Continent and of Wallace, Keitt and Jones and other workers in the United States, that almost incredible numbers of ascospores may be discharged from overwintered dead scabbed leaves. Wallace has computed that from a fragment of leaf

1 cm. square 5630 ascospores may be discharged in 45 minutes. It has been found that all through the spring months, whenever rainy spells set in, ascospores are forcibly discharged from the leaves on the ground. Keitt and Jones found, by drawing the air into an apparatus and filtering, that in one orchard, on a rainy day in May, the number of ascospores per cubit foot of air averaged 289 throughout a 5-hour period. Observers in the United States are agreed that dead scabbed leaves provide the main source of primary infection each season. There is little doubt, I think, that, in this country also, such dead leaves provide the main source on the farm. In our spraying experiments in Kent we have almost always been able to find in the orchard at the time when the first spraying was being carried out, dead leaves with ripe perithecia, which on being brought into the laboratory and kept wet soon discharged spores.

Experiments in trying to prevent the formation of the perithecial stage or to prevent the discharge of ascospores have recently been carried out. We may instance the work of Dr Keitt and his co-workers in the United States and of Dr Curtis in New Zealand. No completely satisfactory results have yet been obtained, but it is obvious that all possibilities should be explored of dealing with what is known to be the chief primary source of infection. At the present time, however, there is no practical method of control in this direction which can be suggested to the farmer. We are driven, then, to rely on spraying to prevent the disease. And, of course, if trees are sprayed thoroughly there should be very little scab present on the leaves, and consequently the formation of perithecia should be largely prevented. And the same applies also to the prevention of scabbed wood by spraying. And this on the whole seems to be the best line to adopt for the control of scab, as the cumulative effects of spraying season after season must inevitably lead towards suppressing the primary sources of infection. But it must be remembered that this method of control depends on the regular annual spraying of the trees, whether they have a crop or not. Have we sufficient scientific evidence on which to advise the grower to spray his orchard irrespective of whether there is a crop or not?

It also depends on whether we can treat the orchard as a self-contained unit from the point of view of liability to scab. There is the possibility of dead scabbed leaves blowing from one orchard to another, but such spread of the disease is probably slight. The spread of scab by secondary infections caused by conidia produced on the leaves is probably confined almost entirely to the individual orchard. The researches of Frey and Keitt have shown that the conidia are not easily detachable in the dry state, but easily become detached in the presence of water. These authors conclude that no important dissemination of conidia can take place in the absence of water, and that though undoubtedly some conidia are dislodged by wind-whipping of leaves, fruit and branches, by contact with wind-blown particles and in other minor ways, the important agency for dissemination of conidia is rain water blown by the wind or dropping from the boughs of the trees.

We can, therefore, I think, look upon the orchard as an independent unit, liable in the main to infection only by spores produced on its own leaves or wood. If so, the case is very different to crops exposed to infection by spores carried on the wind from outside sources. Support is thus given to the view that it may prove feasible by adopting a programme of routine annual sprayings to eliminate or greatly reduce the primary source, or sources, of infection in any one orchard.

Spraying; the best fungicide. Against apple scab we have practically only two fungicides of proved value; home-made Bordeaux mixture and lime-sulphur (with or without arsenate of lead). Besides these, there are the so-called colloidal sulphurs and ready-made Bordeaux pastes. In our experiments Bordeaux paste has proved to be definitely inferior to home-made Bordeaux mixture. It can safely be said, I think, that on the scientific evidence at present available "colloidal" sulphur cannot be recommended as a main spray for the farmer. There is, of course, the question of "dusts" or "dry spraying." Dry mixtures of finely divided copper sulphate and quicklime and preparations of sulphur as powders—including proprietary articles such as "Kolodust," are coming into use. No scientific experiments in this country have yet, I think, demonstrated that dusts give as effective a control of scab as wet sprays. In view, however, of the favourable results obtained abroad in the hands of some, though not all, experimenters, dry spraying against apple scab merits serious attention in this country, although, I believe, it cannot at present be recommended with confidence to the farmer.

We come back, therefore, to home-made Bordeaux mixture and lime-sulphur, as the main sprays which can be recommended to the farmer. Each of these sprays has its own special advocates. Those who advocate Bordeaux mixture believe that it is the stronger and more adherent fungicide; that it is safe to use on certain commercial varieties, without fear of causing injury; and they point out that cases are known in this country where lime-sulphur has caused a "drop" of 40 per cent. or more of the young apples; that certain experimenters abroad, after having given up Bordeaux mixture in favour of lime-sulphur, have returned to the former, and quote Sanders, who gave as his opinion in 1924 that in Nova Scotia spraying with lime-sulphur had been responsible over a period of seven years for a loss of at least seven million barrels of apples. Those who advocate lime-sulphur may reply that the making of Bordeaux mixture is too difficult an affair for the average farmer; that cases are known in this country where serious russeting and leaf fall have been caused by Bordeaux mixture, and that many experimenters abroad report the same thing—and may quote the statement recently made by Keitt and Jones, founded on experiments carried out in Wisconsin on five commercial varieties of apples and extending over six consecutive seasons that Bordeaux mixture proved unsatisfactory commercially because of russeting, inferior finish of fruit and foliage injury, and that lime-sulphur, 1–40, appeared to be the most satisfactory spray.

We at Wye are to be ranked among the advocates of home-made Bordeaux mixture. In the past it was impossible to get the average farmer to make Bordeaux mixture from quicklime; as Pickering observed in one of the Woburn Reports, the obtaining of quicklime and the simple operation of slaking it with water is too much for the farmer. Perhaps the farmer is right—the time required may be too precious in that busy time of the year when spraying has to be done. But now that it has been shown that equally good Bordeaux mixture can be made with slaked lime (calcium hydrate), which can be obtained cheaply, of reliable purity, in paper-lined bags, the operation of making Bordeaux mixture presents no difficulty whatever, and is in fact now being carried out by bailiffs and foremen on fruit farms, and also on farms where hops have to be sprayed against Downy Mildew. In our experiments we have used Bordeaux mixture on certain varieties of apples without the occurrence of any injury of commercial importance—provided, of course, that trees are not over-

sprayed. Such varieties are Bramley's Seedling, Newton Wonder, Allington Pippin and, perhaps, Worcester Pearmain. On Newton Wonder and Allington Pippin we have obtained consistently good results for the last three years. So far so good, for the seasons concerned, but the cautious pomologist is likely to remark: "Wait—and the season will come when you will find Bordeaux mixture causing serious russeting on these varieties." This caution may be necessary; we do not know. At any rate, before a definite routine spraying programme can be confidently recommended to the farmer, it must be based on the results of experiments carried out over a considerable number of consecutive seasons.

Lime-sulphur used on Bramley's Seedling has given with us as good a control of scab as Bordeaux mixture. But for the warning given by the East Malling Research Station of the possibility of injury in the form of the "drop" of the young apples, the use of lime-sulphur might be more advocated. Possibly it will be found a good plan to use Bordeaux mixture for the early pre-blossom spraying, and lime-sulphur for later sprayings.

We are of the opinion that neither Bordeaux mixture nor lime-sulphur requires the addition of any "spreader." With the use of sufficient pressure and the right type of nozzle, the very fine misty spray secured will effectively cover the whole leaf surface. It may be noted that Keitt and Jones reached the conclusion that the addition of gelatine or glue to lime-sulphur appeared to decrease rather than increase its effectiveness, and that the addition of casein-lime to Bordeaux mixture and lime-sulphur made no significant difference[1].

The time for spraying and the number of applications. In the control of apple scab nothing is of greater importance than to choose the right time for spraying. Keitt and Jones emphasise the fact in the conclusion drawn from their series of spraying experiments: "it was apparent that timeliness of application was more important than the material used." According to them, Wallace was the first, in 1894, to advocate a pre-blossom spraying, viz., at the "pink bud" stage. In 1926 Keitt and Jones reported that experiments showed that two pre-blossom applications were necessary on badly scabbing varieties. In four of the six years of experimentation the pre-pink application greatly increased scab control; in two seasons it was of little or no value. The meteorological data collected showed that in these four seasons early spring rains of sufficient number and duration led to serious scab infection prior to the application of the spray at the pink bud stage. It is held further by the same authorities that under severe epidemic conditions three applications during the pre-blossom period may be necessary: (1) at the "green tip" stage; (2) at the "closed cluster" stage; (3) at the "open cluster" or "pink bud" stage. If only two pre-blossom applications are to be given, the most critical periods appear to be the "green tip" stage and just before blossoming, preferably the pink bud stage.

The observations quoted above all refer to the problem as it exists in the United States. We have to work out our own salvation in this country, but it seems clear that our problem is much the same. Until quite recently there was no pre-blossom spraying in this country—I think the recognition of the necessity for spraying at the pink bud stage was due to our recording, in 1924 and 1925, the common occurrence in orchards of the perithecial stage and the primary infections which occur on young

[1] In recent experiments at Wye it has been shown, in tests with the hop powdery mildew, that the addition of gelatine to lime-sulphur reduces the fungicidal value.

leaves before the blossom opens. At the present time it is becoming usual to adopt a programme of three applications; the first, at the pink bud stage; the second, immediately the petals have fallen; and the third, two to three weeks later. On certain varieties under certain cultural conditions this programme will secure a very efficient control of scab. Instances of such control have been given by many workers, and we will give one from our experience. The plantation sprayed consisted of 14-year-old trees of Allington Pippin and Newton Wonder, situated on the farm at the College, Wye. We will confine ourselves to the results obtained on the Allingtons, but it may be mentioned that strictly similar results were obtained on the Newtons. The sprayed trees consisted of two blocks of 12 trees each, and the control (unsprayed) trees were 12, in three plots which were distributed among the sprayed trees. Three applications of the fungicide used were made; the first, at the pink bud stage; the second, as soon as the petals had fallen; the third, two to three weeks after the second. The strength of the Bordeaux mixture was copper sulphate, 8 lb.; quicklime, 8 lb. or hydrated lime, 12 lb.; water, 100 gals. The lime sulphur was used at the strength 1 to 60. The entire crop was graded by hand into three grades; Grade 1 comprised apples "commercially" free from scab, *i.e.* the apple was either entirely free, or it bore not more than three very minute spots of scab, each not larger than a pin's head; in Grade 2, the apple was obviously affected, the scab spots being few or many, but the fruit was not unmarketable; in Grade 3, the apples were so cracked or disfigured by scab as to be unmarketable. The results obtained for the past three years are shown in Table I.

Table I.

Allington Pippin.

Year	Treatment	Grade 1	Grade 2	Grade 3	Crop ton	Crop cwt.
1927	*A.* Hydrated lime Bordeaux	89	11	0	4	2
	B. Quicklime Bordeaux	88	12	0		
	Control	6	77	17		
1928	*A.* Hydrated lime Bordeaux	89	11	0	0	14½
	B. Quicklime Bordeaux	88	12	0		
	Control	15	75	10		
1929	*A.* Hydrated lime Bordeaux	87	13	0	3	18
	B. Hydrated lime Bordeaux followed by lime-sulphur twice	91	9	0		
	Control	23	72	5		

It is an interesting fact to find that for three consecutive seasons a programme of three applications of Bordeaux mixture secured an excellent control of scab, converting crops which, on the unsprayed trees, contained only 6 to 23 per cent. of clean apples to crops of which 87 to 91 per cent. were clean. No russeting or other spray injury of any moment occurred. It is to be noted that while the seasons of 1928 and 1929 were hot and dry, that of 1927 was wet.

Under the conditions of the experiment, then, this spraying programme, with careful but strictly commercial spraying, using the spraying rod, satisfactorily controlled scab, whether the weather was wet or dry, on trees of Allington Pippin and Newton Wonder in a plantation.

Sometimes, however, under other conditions and with a different variety, control of scab is not secured by these three sprayings. Let me give an illustration of this.

The trees used were in an orchard on a farm near Canterbury, and of the variety Worcester Pearmain, about 24 years old. Both the sprayed plots, which contained 138 trees, received quicklime Bordeaux mixture (8 : 8 : 100); the first plot was sprayed three times (at the pink bud stage and two post-blossom applications), and in the second plot, the spraying at the pink bud stage was omitted. There were two control plots, at opposite ends of the orchard, containing in all 70 trees. The expectation was that control of scab would be secured on the first plot but not on the second. Nothing of the kind happened, however, as will be seen on reference to Table II.

Table II.

Worcester Pearmain.

Year	Treatment	Grade			Crop	
		1	2	3	ton	cwt.
1927	(1) Bordeaux thrice	55	45	0	6	7
	(2) Bordeaux twice	61	39	0		
	Control	30	69	1		
1928	(1) Bordeaux thrice	3	54	43	1	11
	(2) Bordeaux twice	3	50	47		
	Control	0	38	62		

In 1927 the control of scab could not be considered altogether satisfactory, as, although the spraying did increase considerably the percentage of clean apples, there was still from 39 to 45 per cent. of scabby fruit. And there was nothing in favour of pre-blossom spraying. But far worse was to follow. In 1928, although the summer was hot and dry, the scab assumed epidemic proportions in this orchard. On the control trees no less than 62 per cent. of the apples were in Grade 3, and the thrice- and twice-sprayed plots gave only 3 per cent. of clean apples and from 43 to 47 per cent. in Grade 3. The spraying programme was obviously quite inadequate under the conditions obtaining here. The biological notes kept by Mr W. M. Ware, in these, as in other, experiments put us on the track of what was probably the main factors concerned. The observations made in this orchard in 1928 showed that at the date of the first spraying (May 2nd) a more or less severe infection of the trees in all the plots had already taken place; as a result of this early infection before the first spraying had been done, not only did the thrice-sprayed plot give no better results than the twice-sprayed, but a programme embracing only one pre-blossom application proved futile. In 1927 Mr Ware's observations showed that, owing probably to the exceptionally dry weather in the spring of that year, in neither of the sprayed plots was there any infection of the leaves before the blossoming period—consequently the pre-blossom application did not affect the control of scab. The other factors which seem likely to have been operative in this failure to secure control of scab are the use of the spray gun instead of the spraying rod, and possibly of cultural conditions, *i.e.* the "pigging" of the orchard and an unusually abundant source of infection in dead scabbed leaves.

In another instance, in 1929, the trees of Worcester Pearmain were in an orchard grazed by sheep and the spraying was carried out with spraying rods. Three applications with hydrated lime Bordeaux mixture were given, and efficient control of scab was secured, the sprayed trees giving 76 per cent. of scab-free apples and the controls only 19 per cent.

It is much to be desired that workers will record those experiments in which the control of scab breaks down. If the necessary biological observations in the orchard are made, some of the factors concerned with the failure may be discovered, such as the presence of unusual sources of primary infections or the occurrence of infections at an unusually early date. Until we secure such *data*, we are not in a position to give the farmer the information for which he is now beginning to ask. It seems clear that under the conditions usually obtaining in this country a pre-blossom spraying at the pink bud stage is necessary and that very often, if this is followed by the application of two post-blossom sprayings, very efficient control of scab is secured; under other conditions, however, which urgently need investigation, more than one pre-blossom application may be necessary. It is interesting to find that some farmers in Kent, as the result of experience, now make a practice of applying two applications of lime-sulphur before the blossoming period. The number of routine sprayings necessary for the control of scab will probably vary in different parts of England.

Accepting as proved the great importance of early spraying before the fungus has established itself on the leaves, a practical difficulty often presents itself. It is a common practice for a number of different varieties to be planted intermixed; if these varieties flower at different times, several visits may be required to catch the different varieties at just the right pre-blossom development. This practical difficulty can only be met by a system of planting blocks of the same variety with adjoining blocks of another variety where cross-pollination is necessary.

It is of course easy for the mycologist, sitting at his writing table, to lay down a spraying programme of pre-blossom applications; it is a very difficult matter for the farmer to carry out this programme satisfactorily under the weather conditions which often prevail here in April and May. But here there can be no compromise. It must be brought home to the farmer that the same weather conditions, viz. frequent spells of rain, which hinder his spraying are just those which provide the necessary conditions for the ejection of countless numbers of spores from leaves on the ground and for primary infections to take place on the opening leaves. The major part of the discharge of ascospores for the season may take place in early spring before the blossoming period. When trees are heavily infected before spraying has been done, it is probably impossible to secure by subsequent spraying a satisfactory control of scab. The farmer must watch the weather, and must have everything ready for spraying—machine, nozzles (and spare parts), spray mixture and gang of men—then on the few fine days that do occur in a wet spring he is able to spray with the knowledge that—in most cases—spraying under such conditions will protect the crop from exceptionally severe attacks of scab.

Spraying machinery and the technique of spraying. It is essential that continuous high pressure is obtained at the nozzle to secure a very fine misty spray which wets the whole leaf surface. In the operation of spraying, either the spray gun or the spraying rod is used. The spray gun (of about 2 ft. in length) gives the choice of a fine or of a coarse spray; when in perfect working order it can be adjusted to give a fine, misty spray and the sprayer can then (if there is not too much wind) walk round the tree and complete the spraying of the lower part of it, using the coarser jet to spray the upper parts of the tree. Not uncommonly, however, owing to the spray gun being slightly out of repair, or in unskilful or careless hands, a coarser spray is used all the time, and it is then only possible for the sprayer to direct very

rapidly the volume of spray *en masse* on to the tree, as any attempt to spray separate parts of it would result in drenching the foliage and probably causing spray injury from overspraying. As a matter of fact the use of spray guns does often result in overspraying. The spraying rod, 6 ft. in length, demands a slower action but is far safer; greater attention can be given to spraying thoroughly every part of the tree, branch by branch being taken without fear of overspraying. Spraying is a skilled operation, and it is more or less an unpleasant one; those farmers are wise who recognise both facts and provide the sprayers with extra payment and with suitable spraying equipment.

Varietal susceptibility and spray injury. I shall merely remark on this subject that, as is well known, some varieties of apples show more or less severe injury when sprayed with Bordeaux mixture, while other varieties are affected injuriously when lime-sulphur or other sulphur preparations are used. The field is a very wide and important one and a great deal remains to be elucidated. The injury liable to be caused by Bordeaux mixture appears to be correlated with weather conditions subsequent to the application.

Besides this type of injury, which may be termed specific to the variety concerned, there is the wide question of injury due to overspraying. Until the art of spraying has been mastered this must remain another problem.

Method of experimentation. It is always necessary to have a considerable number of control trees left unsprayed; the control plots should if possible be situated in different parts of the sprayed orchard, as the incidence of scab is liable to vary in different parts of an orchard. Owing to the drift of fine spray that is bound to take place at the different times of spraying, either guard rows should be established, or the entire crop should be collected from all the trees and graded. Hand grading for scab is of course absolutely necessary; it is impossible to gauge accurately by the eye the results of spraying by inspecting the crop on the trees. The farmer may go astray by relying on this method of judging. In our experiments we have counted the number of apples in each grade and also have weighed the apples in each grade. We have found that the percentage by number in each grade approximates very closely to the percentage by weight.

A biological examination of the sprayed and unsprayed plots should be made at short intervals throughout the season for the noting of such points as the time of the occurrence of the primary and secondary infections, the killing or non-killing of scab on the sprayed parts, the occurrence of fresh infections on fresh growth, and the first appearance of any spray injury. Such observations may furnish the clue to the solution of many problems. Unfortunately the experimenter too often lacks the time to be able to pay many such visits of observation, and can do little more than spray the trees and grade the crop for scab. I am convinced, however, that we shall not get the problem of the satisfactory control of scab on the different varieties in different parts of the country solved until more trained workers carry out such observations in sprayed orchards.

I would like to conclude with a plea for the recognition of the fact that the economic control of apple scab has become a matter of national importance. There is a colossal waste of English apples through scab every season; the loss to farmers must run into many hundred thousands of pounds. There is now no commercial variety immune from scab such as Bramley's Seedling was in the past. Every year fresh

apple orchards and plantations are coming into bearing; only with rare exceptions are the trees sprayed against scab in a rational manner. A foreigner looking at the English apples displayed in the shops in any of our country towns would be driven to conclude that the "National Mark" of the English apple is a scab spot! On the other hand, farmers are showing an increased interest in the question of spraying against scab; the advisory mycologist meets an increasing number of farmers who ask for the spraying programme to adopt for growing clean apples only. There is a twofold task: (1) of advising the grower how to control scab and of giving demonstrations of the method so far as it is known; and (2) of carrying out investigations to solve the many problems which must be solved before the control of scab is possible on many varieties under many cultural conditions. It can hardly be claimed that this work is being done on a scale commensurate with its national importance. I would put in a plea for apple scab to be treated by the official authorities as virus diseases now are. The Virus Committee of the Ministry of Agriculture finds ways and means to secure investigations into virus diseases and their methods of control. Could not an official Apple Scab Committee function in the same way?

II. Mr F. R. Petherbridge.

Mr Petherbridge said: In the Eastern Counties Apple Scab is now one of the most important factors in the profit and loss of apple growing. During the past three years, in conjunction with Messrs Dillon Weston and Kent I have carried out spraying trials on the same trees. The variety used was Worcester Pearmain and the trees which were planted 22 ft. by 24 ft. were over 20 years old, and in the past had been badly attacked by "scab" but were comparatively free from "capsid" and "red spider." To prevent interference from attacks of aphides and caterpillars all the trees were sprayed each winter with a "tar-oil" wash.

The spraying. Two barrow-type hand-power machines each with one lance (bent near the nozzle) were used and the nozzles were adjusted to give a *very fine mist-like* spray. The spraying was very carefully carried out and practically the whole surface of the trees was covered with the fungicide. This spraying was more thorough than is normal in commercial orchards.

The spray fluids used were lime-sulphur and home-made Bordeaux mixture, made on the excess lime formula (10 lb. lime, 3 lb. copper sulphate, 40 gallons water). Since it is usual in commercial practice to add arsenate of lead to fungicides, 5 lb. of lead arsenate paste were added per 100 gallons of each of the diluted sprays. Every year the lime-sulphur was used at a strength of 1 in 30 for pre-blossom spraying and at a strength of 1 in 60 for post-blossom spraying.

Each plot consisted of eight trees. Two plots were sprayed with lime-sulphur and two with Bordeaux mixture. Every sprayed plot was next to a sprayed plot on one side and to a control plot on the other, necessitating three control plots. Each year all the fruit from the trees was sorted into the following grades:

(*a*) Free from scab.
(*b*) Showing slight scab spots, the total scabbed area being less than could be covered by a sixpence.
(*c*) Badly scabbed and seriously affected in market value.

1927	Pre-blossom spray	May 2nd
	Post-blossom spray	May 23rd
1928	Pre-blossom spray	May 1st
	Post-blossom sprays	May 21st and June 15th
1929	Pre-blossom spray	May 9th
	Post-blossom sprays	May 30th and June 20th

The results obtained are shown in the following table:

Percentage weight of apples.

Spray	Year	Clean	Slightly scabbed	Badly scabbed	Average crop per tree in lb.
Control	1927	0·2	7	93	57
	1928	0·24	10	90	41
	1929	51	42	7	104
Lime-sulphur 1 in 30 pre-blossom 1 in 60 post-blossom	1927	6	44	50	119
	1928	30	50	20	135
	1929	91	7	2	242
Excess Bordeaux	1927	48	42	9	107
	1928	72	21	7	118
	1929	98 (14 % russeted)	1·6	0·4	205

Half-strength excess Bordeaux gave results almost as good as those of the excess Bordeaux mixture.

The apples were marketed chiefly in boxes, a few being sent in trays. The monetary returns (less the cost of packages, freightage and commission) works out as follows:

Average returns per acre.

	Average of 1927–8	1929*	Average for 3 years
Control	£14. 10*s*. 0*d*.	£60	£30
Lime-sulphur	£67. 10*s*. 0*d*.	£160	£98
Bordeaux	£80. 0*s*. 0*d*.	£135	£98

* Not based on actual returns as in 1927 and 1928 but on £20 per ton for 1st grade and £14 per ton for 2nd grade.

These figures show that there is a very little profit on the unsprayed trees and a profit of over £60 per acre on both the lime-sulphur and Bordeaux sprayed trees.

They also show that the intensity of apple scab is very variable on the same trees. In 1927 and 1928 the attack was very severe and practically the whole of the apples on the unsprayed trees were scabbed. In the dry season of 1929, on the other hand, over 50 per cent. of the fruit on the same trees was clean.

The yield on the sprayed trees as a result of the first year's spraying was about double that on the unsprayed trees, and after the second year's spraying it was about three times as big. An examination of the trees during the winter following the second spraying showed that the sprayed trees had much better prospects of a good crop the following season, judging by the number and size of the fruit buds. In spite of the comparatively small amount of scab in 1929, the yield on the sprayed trees was again double that on the control trees.

The lime-sulphur plots gave the highest yield, and the apples on these plots coloured earlier than those on the other plots.

In 1927 and 1928 there was a slight russeting of the fruit on the Bordeaux trees, and in 1929 this was more noticeable, 14 per cent. of the fruit being russeted, and in addition there was some scorching of the older leaves which caused a number of them to fall from the trees.

Worcester trees over 20 years old, in the eastern counties, are often regarded as being unprofitable. The above experiment shows that scab is capable of bringing about this state of affairs, but that it can be remedied and a big profit obtained if the trees are sprayed with either lime-sulphur or Bordeaux mixture.

It also demonstrates the cumulative effect of spraying, as, after two years' spraying it was easy to pick out the sprayed trees during the dormant season by their healthier appearance. It also shows that tar-oil spraying alone is of little value on a variety like Worcester when scab is prevalent.

A comparison of lime-sulphur and Bordeaux mixture. Bordeaux mixture gave a better control of scab than lime-sulphur, but lime-sulphur gave a bigger crop than Bordeaux.

Lime-sulphur also gave better coloured fruits, especially in 1929, when the Bordeaux mixture caused a fair amount of russeting.

Since the introduction of tar-oil washes, "red spider" (*Oligonychus ulmi*) has been a serious pest of apples. This is controlled by lime-sulphur spraying but not by Bordeaux mixture.

With some varieties it is not advisable to use lime-sulphur after blossoming if it has not been used on the same trees before blossoming. Varieties like Lane's Prince Albert may suffer severe defoliation if trees not sprayed before blossoming are sprayed after blossoming with 1/100 lime-sulphur.

Bordeaux mixture is a standard article but not easy to make in the field. Lime-sulphur is a proprietary article and easy to make, but owing to its proprietary nature is liable to vary considerably. Four samples carefully taken in 1929 gave the following analysis:

Lime-sulphur solutions.

	(a)	(b)	(c)	(d)
Specific gravity	1·306	1·2995	1·2321	1·1944
Total sulphur	33·83	32·76	24·46	17·52
Polysulphide sulphur by modified Chapin method	26·28	25·47	17·91	11·31

Results expressed as percentages $\frac{\text{weight}}{\text{volume}}$, *i.e.* pounds in 10 gallons.

These differences probably explain the varying results obtained by different growers.

Varieties vary very considerable in their susceptibility to scorch from Bordeaux and lime-sulphur. It is important that those who advise growers should know how the different varieties usually behave. For this purpose we have (in conjunction with the County Horticultural Officers) drawn up a table for the use of growers in the Eastern Counties. Our knowledge of some of the varieties included is somewhat limited and further observations may necessitate alterations.

First application. Pink stage.

All varieties. Bordeaux or lime-sulphur (1 in 30) plus lead arsenate. Varieties in class (*a*) to be sprayed after blossoming with lime-sulphur should be sprayed before blossoming with lime-sulphur.

After blossom spraying.

(*a*) 1 in 100 lime-sulphur plus 0·3 per cent. lead arsenate paste.

Cox's Orange Pippin.
Rival.
Lane's Prince Albert.
Wellington.
Blenheim Orange.
Lord Grosvenor.
J. Grieve.
Beauty of Bath.
Chas. Ross (not usually necessary to spray).

(*b*) 1 in 80 lime-sulphur plus 0·5 per cent. lead arsenate paste but not Bordeaux.

Newton Wonder.

(*c*) 1 in 60 lime-sulphur plus 0·5 per cent. lead arsenate paste but not Bordeaux.

Allington Pippin.
Lady Sudely.
Gladstone.
Duchess Favourite.
Lord Derby.
Annie Elizabeth.
King Edward.

(*d*) 1 in 60 lime-sulphur or excess Bordeaux plus 0·5 per cent. lead arsenate paste.

Worcester.
Bramley.
Bismarck.
Emneth Early (Early Victoria).

Stirling Castle. Before blossoming twice with excess Bordeaux. No after blossom spraying.

Dusts have now found a place in the control of scab in parts of America.

Our experiments on these are mainly confined to 1929 when the incidence of scab was slight. Six dustings with a proprietary sulphur dust gave very similar results to three sprayings with lime-sulphur.

If, in normal seasons, dustings will give almost as good results as spraying it will find favour with growers, as it is easily and quickly applied and is economical of labour.

III. Mr M. H. Moore.

Mr Moore said: It is my intention to outline briefly the main results obtained at East Malling in experiments on scab control during the past three years, and to indicate the methods employed in obtaining these results. The subject will be treated under three chief heads, viz. the effect of spraying, the effect of manuring and the effect of rootstock.

1. The effect of spraying.

The spraying trial plot at East Malling was originally used as a trial of Malling "Paradise" rootstocks, but when the scab work was undertaken, this plot was given over to the spraying experiments.

Two varieties, Cox's Orange Pippin and Stirling Castle, are planted in alternate rows, and the trees are worked on various layered rootstocks. We shall not be concerned with Stirling Castle as this is used in another experiment, so that all the *spraying* work dealt with now has been carried out on the former variety.

There are about 160 trees and 11 different rootstocks, there being in almost every case 16 trees on the same rootstock. Each set of 16 trees has been divided up so that 5 are sprayed with one fungicide, 5 with another and 6 are controls, that is, they have not been sprayed with a fungicide.

All treatment apart from spraying is identical as far as is possible—cultivation, manuring, pruning. The trees are now about eight years old and are in good condition, the first satisfactory crop being picked this year (1929). The spraying is done by means of knapsacks. The preparation and mixing of the sprays are done as carefully as possible, and the application is made under personal supervision. Large hessian screens are placed around each tree while it is being sprayed to prevent spray drift from settling on other trees. Three sprays are used:

(*a*) *Bordeaux mixture*. 8–8–100 (burnt lime) before blossoming, and "excess-lime," 8–25–100, after blossoming. In 1929 hydrated lime was used instead of burnt lime at the rate of 8–12–100 before and after blossoming, with disastrous results.

(*b*) *Lime-sulphur*. 1–30 before blossoming and 1–100 after blossoming, although in one experiment the strength after blossoming is reduced to 1–150.

(*c*) *Colloidal sulphur*. 4 lb. in 100 gallons, after blossoming only. Gelatine is used in this case as a spreader.

Lead arsenate is included with the majority of the sprays, in order that the experiments shall not be interfered with by attacks of caterpillars and other biting insects. In such cases, the control trees are sprayed with lead arsenate only.

The main spraying trial is subdivided into a number of experiments to test the effect of omitting the pink bud spray, or the two post-blossom applications, compared with that obtained by the application at all three periods, the number usually recommended in this country as the complete scab spraying schedule.

Methods of obtaining results.

(*a*) *Scab on the young shoots* (*current year*).

During the three years from 1926, various methods have been tested, all having as the basic object in view the expression of the area of scab on the shoots in figures which would be comparable, tree by tree. The method has been made less laborious each year, of necessity, as the trees were getting bigger and more young wood was being produced. The final method, which is the one now used, will be described here.

The prunings from every tree are collected separately and weighed as soon as possible, and the approximate area of scab on them is estimated in the following manner: Certain shoots are selected as types, and these bear areas of scab (graded by inspection), so chosen that each shoot has approximately twice as much scab as that representing the grade next below it. These areas of scab are maxima for each grade, and all the shoots from each tree separately are graded into those categories, which, for the purpose of obtaining comparable figures ultimately, are numbered 1, 2, 4, 8, 16, 32, etc. Shoots which are quite free from infection are placed in a grade by themselves, this grade being represented by a category number O.

When all the shoots from a tree have been graded, the number in each category is ascertained and multiplied by the corresponding category number. The products are then added. In order that the area of the tree open to infection shall be standardised as far as possible, the sum thus obtained is made the numerator of a fraction of which the denominator is the weight of the prunings, this latter being regarded as the most convenient, quick measure of the relative surface area of the twigs. To avoid decimals, the fraction is increased 100 times, but the result is evidently not a percentage. The final figure has no intrinsic value, but is regarded merely as a convenient means of comparing the scab infection on different trees, and ultimately for obtaining in comparable figures a measure of the control of scab given by different spray treatments. It represents amount of scab per unit weight of wood.

Example: Row 1. Tree 5. Cox's Orange Pippin on Type IV.

Categories	O	I	II	IV	VIII	XVI	XXXII	
No. of shoots	167	68	11	16	12	13	0	
Sum of products	0	68	22	64	96	208	0	=458

Wt of prunings = 52 ounces.

"Scab equivalent" for the tree = 458/52 × 100 = 881.

This so-called "category method" is the chief basis on which all the results have been obtained for infection on the shoots, leaves and fruits, and also for the estimation of spray damage such as fruit russeting and leaf scorch.

Results (*on shoots*). One application, in the pink bud stage only, of Bordeaux mixture and of lime-sulphur respectively, has been followed by a pronounced check to infection by scab in the case of both fungicides. The effect obtained demonstrates the great value of spraying with a good fungicide at this period. There is no constant indication that one spray was better than the other.

Two applications after blossoming (one at petal fall and the other about three weeks later) have given very good control of infection, and in this case Bordeaux mixture is more efficient than lime-sulphur. It will be remembered that the strength of the latter spray is considerably reduced for post-blossoming applications, and its fungicidal value is undoubtedly thus impaired.

Three applications, one before and two after blossoming, have given excellent control, the beneficial effect of the inclusion of the pink bud application being evident. Bordeaux mixture was again better than lime-sulphur.

Some control has been given by two post-blossoming applications of the weakest lime-sulphur (1–150) and of colloidal sulphur, there being no difference in efficiency between the sprays.

(*b*) *Scab on leaves.*

The trees were inspected at three or four distinct periods throughout the summer and estimations were made, by inspection, of the amount of scab on the leaves. First of all, a general impression of the severity of scab on the plot was obtained in order to be able to gauge the differing severity of infection and to fix definite categories in the mind's eye. Each tree in turn was carefully inspected for scab on the leaves, all leaves coming under a general inspection. It was then allotted a category number according to the severity of scab infection, and the numbers were averaged for trees in each spray treatment.

Results. One pre-blossoming application has checked infection by approximately half, Bordeaux mixture and lime-sulphur being equally efficient.

Two post-blossoming applications of Bordeaux mixture and of lime-sulphur have yielded better results than the single pre-blossoming application, the former fungicide being more effective.

Three applications have given excellent control, Bordeaux mixture being again somewhat more efficient.

Slight control has been given by two post-blossoming applications of lime-sulphur (1–150) and of colloidal sulphur respectively, the effects being similar.

(*c*) *Scab on fruit.*

The method of grading the fruit and working out the results was precisely similar to that described for the shoots, with the exception that the denominator of the fraction was the number of apples and not the weight. A great saving of time is thus effected on a large quantity of fruit.

Results. One application only, in the pink bud stage, has given surprisingly good control of scab, there being little difference in effect between Bordeaux mixture and lime-sulphur.

Two post-blossoming applications have also given good control, but on the whole this is little, if any, better than that afforded by the pre-blossoming spray. In the case of the shoots and the leaves, the first application alone was not usually as efficient as the two later ones. The fungicides form a protective covering over the new growth, but when the application is made only in the pink bud stage, the new shoots and most of the new leaves are still in the bud and so would not receive the protective covering. On the other hand, potential fruits are present at the pink bud stage, the receptacle becoming the apple. Observations each year show that at this period scab infection is very prevalent, and, in cases of bad attack, the flowers often become severely infected on the pedicel, receptacle and calyx, and even the petals, and the young fruits consequently do not escape. When the trees are efficiently sprayed before blossoming this severe infection does not occur, but when the first application is not made until after blossoming, scab has already become established on the flowers and young fruitlets, thus considerably prejudicing the chances of post-blossoming applications alone of getting the upper hand.

The beneficial effect of a pre-blossoming application is again evident in the results following three applications. Here excellent control has been given, Bordeaux mixture being somewhat more efficient than lime-sulphur.

Two post-blossoming applications of lime-sulphur (1–150) and of colloidal sulphur respectively, have given only slight control, there being little difference in effect between the sprays.

Effect on the tree (spray injury).

(*d*) *Leaf fall.*

In order to obtain a measure of any leaf fall caused by the sprays, hessian troughs were erected around representative trees immediately after the application of the petal fall spray. These troughs were visited at intervals of a few days and the number of leaves in each counted and recorded. In 1929, however, it was found that the trees had outgrown the troughs, which had also, for the most part, rotted. The method during this year was therefore modified, and counts were taken of the leaves

underneath the trees. Though this was not so accurate as the trough method, it was sufficiently good to show big differences.

Results. Lime-sulphur at the strengths used caused no leaf fall. "Excess-burnt-lime" Bordeaux mixture after blossoming was followed by slight leaf dropping, but in 1929, when hydrated lime was used in the Bordeaux at the rate of 12 lb. to 100 gallons, very severe injury was the result, the trees being almost completely defoliated. Colloidal sulphur caused no leaf fall.

(*e*) *Leaf scorch.*

This was estimated in the same manner and at the same periods as scab on the leaves.

Results. Lime-sulphur (1–30) before blossoming caused negligible scorching of the margins of the first leaves. After blossoming, the weak (1–100) solution caused no leaf scorch. "Excess-burnt-lime" Bordeaux mixture after blossoming was followed by a moderate amount of spotting of the older leaves, and there was rather too much of this to be negligible. When hydrated lime was substituted for burnt lime in 1929, the spraying was followed by very severe scorching. This was probably due either to an inferior sample of lime or to the use of insufficiency of it to produce a "safe" Bordeaux. The figures indicate that lead arsenate has probably caused slight leaf scorching, but colloidal sulphur has not.

(*f*) *Fruit russeting.*

The fruit was graded for fruit russeting, and figures were obtained by the "category" method as for scab on the fruit.

Results. Lime-sulphur, colloidal sulphur and lead arsenate caused no fruit russeting. Bordeaux mixture caused severe fruit russeting whenever it was applied in these experiments, even when the application was made only in the pink bud stage. This spray cannot be recommended for use on Cox's Orange Pippin on account of the injury caused.

(*g*) *Fruit drop.*

At first, this form of injury was gauged by recording the number of fruit set and the number picked and by subtracting the two figures to get the number of fruit dropped. In 1929, the number of fruit actually fallen was recorded.

Results. Bordeaux mixture has caused a fruit drop when applied after blossoming. There is also evidence that when this spray was applied in the pink bud stage only, some damage was caused to the blossoms and young fruitlets. These results are based chiefly on the number of fruit picked. There is no evidence that lime-sulphur or lead arsenate caused fruit drop. Though there was a loss of fruit from the arsenate-sprayed controls, it is thought that this was due primarily to infection by scab.

2. The effect of manuring.

For several years, two plots of trees of the varieties Worcester Pearmain and Bramley's Seedling, "worked" on various Malling Paradise rootstocks, have been used in an experiment designed to show the effect of manuring on tree performance. All treatment such as cultivation, spraying and pruning has been as far as possible identical on the two plots, and, for the purposes of the manurial experiment, one plot is a replica of the other.

The "starved" plot has received nothing but green manure such as mustard, cabbage and rape, grown on the plot and ploughed in, since 1920. The other plot has had what one might call balanced manuring, or complete manuring, as far as the needs of the trees are known. Dung, shoddy, meat, bone and fish meal and potash have been applied.

There was a large crop of fruit in 1929, so samples from each tree were taken and graded for scab by the "category" method previously described.

Large differences were found between the amount of infection on the manured plot and that on the unmanured plot, even though all trees had received two applications of lime-sulphur at the same period during that year.

The fruits from the "starved" trees were very much more heavily infected than were those from the manured trees, and the results lead to the conclusion, from the one year's figures, that infection by scab is very considerably checked on trees that receive balanced manuring. This fact in all probability accounts in some measure for the diversity of results obtained by different growers, even though using the same scab-spraying schedule, and emphasizes the necessity for consideration of all possible factors in attempting to interpret such confusing experiences.

3. The effect of rootstock.

Up to the present, data have been obtained on three varieties—Cox's Orange Pippin, Worcester Pearmain and Bramley's Seedling—which show that rootstock has an influence on the degree of infection by scab.

Generally speaking, Cox's Orange Pippin on Malling Nos. I and IX respectively was much more susceptible to scab than it was when worked on either No. XV or No. XIII.

One year's results have shown that Worcester Pearmain was much more heavily infected with scab in 1929 on Malling No. IV than on No. V. Bramley trees also on No. V were only comparatively lightly infected.

An interesting case was provided by Bramley's on No. I. On the manured plot these trees were more heavily infected than those of the same variety on other stocks, while on the unmanured plot they showed the lowest degree of infection in the Bramley series, though the actual amount of infection was greater on the unmanured plot than on the manured.

Emphasis can conveniently be laid here on the necessity for the standardisation of trees, etc., used in experimental work of this nature.

[A number of lantern slides were shown, illustrating by tables and photographs the various points raised during the address.]

REPORT OF THE COUNCIL OF THE ASSOCIATION OF ECONOMIC BIOLOGISTS FOR THE YEAR 1929

During 1929 the Association has met on eight occasions. On six of these various subjects of interest were brought before the Association by members and visitors, to whom it is greatly indebted. The subjects included Empire Marketing, Virus Disease Research, Australian Agricultural Problems, Dairying Research, Research on Stored Products, and Apple Scab Investigations.

In May the Association had the privilege of visiting the London Docks by courtesy of the Port of London Authority.

The Summer Meeting was held at Cambridge, and the Association is indebted to members of the University for the hospitality and courtesy received during the meeting. The attendance, including visitors at meetings, has varied from 49 to 70 with an average of 58, which shows an increase on last year.

One Honorary Member, Professor Vavilov, was elected.

Thirty-five new members have been elected during the year. There have been five resignations and one death, and the Association now numbers 289 Honorary and Ordinary Members.

During the past year the Association has again enjoyed the hospitality of the Botany Department of the Imperial College of Science and Technology for their meetings. The Council feel sure that the Association will approve of recording its grateful thanks for this privilege.

Papers read to the Association during the year 1929.

Jan. 25th. Mr S. G. Tallents: "On the Work of the Empire Marketing Board."

Feb. 22nd. Dr K. M. Smith: "Some Experiments on the Insect Vectors of Virus Diseases of Potatoes."

Mar. 15th. Sir John Russell: "Agricultural Problems in Australia." Mr F. L. MacDougall: "The Commonwealth Council of Science and Industry."

Oct. 25th. The Work of the National Institute for Research in Dairying. Dr R. Stenhouse-Williams: "General Account." Mr W. L. Davies: "Fishiness in Dairy Produce." Mr G. M. Moir: "Economic Aspects of the pasteurisation of Milk for Cheese-making." Messrs Blissett and Little: "The Need for Further Knowledge concerning Parasitic Diseases in Pigs." Mr J. G. Davis: "Vitamin B and an Anaerobic Pigment producing Organism." Messrs Golding and Halkett: "The Manuring of Pastures with Sulphate of Ammonia."

Nov. 22nd. Research on Infestation of Stored Products. Mr W. S. Thomson: "Survey and Intelligence Work." Mr G. V. B. Herford: "Biological Work." Mr R. H. Bunting: "Infestation of Stored Products by Moulds."

Dec. 13th. The Incidence and Control of Apple Scab. Prof. E. S. Salmon; Mr F. R. Petherbridge; Mr M. H. Moore.

REPORT OF THE HONORARY TREASURER FOR THE YEAR 1929

For the year ending December 31st, 1929, current subscriptions received amounted to £322. 2*s*. 0*d*., this being an increase of £49. 6*s*. 0*d*. on the previous twelve months. Arrears of subscriptions amounting to £16. 5*s*. 0*d*. were paid, while the sum of £23. 15*s*. 0*d*. remained owing at the end of the year with respect to contributors two years or less in arrears. This latter amount, it may be added, is less than that for the two previous years and it is hoped that the members still in arrears with payments will assist the Association by discharging their debts.

The working expenses of the Association, apart from the *Annals of Applied Biology*, amounted to £33. 0*s*. 0*d*. as compared with £39. 3*s*. 6*d*. for the previous year. The publication account of the *Annals of Applied Biology* showed a reduction of £93. 8*s*. 0*d*., notwithstanding 600 copies were printed as compared with 500 in previous years. This is largely due to the volume being of somewhat smaller dimensions and to an increase of £55. 12*s*. 0*d*. in non-members' subscriptions. After net receipts from sales, etc., amounting to £587. 0*s*. 5*d*., had been deducted, the balance due to the publishers for Volume XVI amounted to £294. 19*s*. 0*d*. The year closed with a cash balance of £83. 1*s*. 5*d*. in income over expenditure for that period. The assets of the Association exceed liabilities by £870. 9*s*. 2*d*., which sum includes a reserve fund of National Savings Certificates of the value of £556. 5*s*. 0*d*.

A. D. IMMS,
Hon. Treasurer.

THE ASSOCIATION OF ECONOMIC BIOLOGISTS

INCOME AND EXPENDITURE ACCOUNT FOR THE YEAR ENDED DECEMBER 31ST, 1929.

Dr | *Cr*

EXPENDITURE.

	£ s. d.	£ s. d.
To *Annals of Applied Biology*:		
Estimated Value of Stock at January 1st, 1929	62 10 8	
Expenditure during 1929	294 19 0	
	357 9 8	
Less: Estimated Value of Stock at December 31st, 1929	60 3 2	
		297 6 6
To Printing and Stationery		11 0 4
Postage and Cheque Stamps		6 13 1
Sundry Out-of-Pocket Expenses of Secretaries and Treasurer		11 2 7
Audit Fee Reserve		4 4 0
Balance, being Excess of Income over Expenditure for the Year		83 1 5
		£413 7 11

INCOME.

	£ s. d.	£ s. d.
By Subscriptions:		
Arrears	16 5 0	
Entrance Fees	17 6 0	
Current	322 2 0	
		355 13 0
By Contributions to cost of papers etc. in *Annals of Applied Biology*		20 0 0
By Interest on National Savings Certificates and Bank Deposit		37 14 11
		£413 7 11

BALANCE SHEET, DECEMBER 31ST, 1929.

LIABILITIES AND SURPLUS.

	£ s. d.	£ s. d.
Sundry Creditors:		
The Cambridge University Press	294 19 0	
Audit Fee Reserve	4 4 0	
		299 3 0
Subscriptions paid in advance		13 15 0
Excess of Assets over Liabilities:		
As Balance Sheet of December 31st, 1928	787 7 9	
Add: Balance of Income and Expenditure Account for 1929	83 1 5	
		870 9 2
		£1183 7 2

ASSETS.

	£ s. d.	£ s. d.
Cash:		
At Bank on Current Account	88 4 0	
At Bank on Deposit Account	455 0 0	
		543 4 0
Debtors for Subscriptions 2 years or less in arrear and considered good		23 15 0
500 National Savings Certificates		556 5 0
Stock of *Annals of Applied Biology*, at estimated value		60 3 2
		£1183 7 2

A. D. IMMS, *Honorary Treasurer*.

We certify that the foregoing Accounts are properly drawn up in accordance with the books, vouchers and documents produced to us, and, in our opinion, the Balance Sheet exhibits a true and correct view of the state of the affairs of the Association.

H. J. COX & CO. *Incorporated Accountants.* } *Auditors.*

HARPENDEN, *January 21st*, 1930.

Vol. XVII, No. 3 August, 1930

THE ANNALS OF APPLIED BIOLOGY

EDITED FOR THE ASSOCIATION OF ECONOMIC BIOLOGISTS

BY

W. B. BRIERLEY

AND

D. WARD CUTLER

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VOLUME XVII AUGUST, 1930 No. 3

THE PHYSIOLOGY OF VIRUS DISEASES IN PLANTS

I. THE MOVEMENT OF MOSAIC IN THE TOMATO PLANT

BY JOHN CALDWELL, B.Sc., Ph.D.
(*Department of Mycology, Rothamsted Experimental Station, Harpenden.*)

(With Plate XXXIII.)

INTRODUCTION.

THE study of virus diseases has, in the last few years, become of great importance. It has been increasingly recognised that many of the common diseases both of plants and of animals are attributable to agents called filter-passing viruses. At present, the main criteria of a virus disease are the symptom-complex induced, its infectivity and the invisibility of the causative agent. The structure of the causative agent is, in the nature of things, not at present recognisable. The general position of virus diseases has been discussed recently by various authors, and reference may be made to their papers for details.

Recently attempts have been made to assess the physical characters (resistance to heat, to alcohol, etc.) of the infective principle and a beginning has been made with the study of the physiological aspects.

As virus diseases are recognisable mainly by their symptoms and their infectivity, these are the two aspects of the problem which have been most studied under laboratory conditions. It is clear, however, that symptoms do not serve as very reliable indices of this type of disease on account of the inherent difficulty of rigidly defining a disease by symptoms which may, under differing environmental conditions, vary between wide limits.

As this uncertainty constitutes a real difficulty, it was decided to attempt, in this laboratory, some more general studies on the physiology of virus diseased plants. Various lines have been opened up, and the one with which this paper deals is that of the movement of the causative agent within the plant. This is an aspect which has been little studied. It was felt, however, to be of considerable importance in that it may be possible to give some indication of the nature of the causative agent if it be definitely shown how its movement through the plant takes place.

Results of previous work on the movement of virus.

The few data relating to this problem are rather scattered through the literature. Bennett (2) was one of the first workers to examine the actual tissues through which movement takes place. He investigated the movement of the curl virus of raspberries through "ringed" stems. From his experiments he concluded that (1) the curl virus can be confined to the inoculated shoot for an indefinite period by the simple process of "ringing," (2) a relatively small amount of "bark" bridging a "ring" is sufficient to permit of the passage of the curl virus from the inoculated part to the parts below the "ring." He suggested that the movement of the virus from the root of the resting stool "may parallel the movement of food."

Some time earlier Severin (14) had attempted to determine the rate of movement of the infective agent. He found that the virus of curly-top of sugar beet moved in "one-half hour at a mean temperature of 103·5° F. through the petiole 7 inches long." This was the quickest rate he obtained. More usually the rate was of the order of 4 inches per hour in his experiments. It was also found that the disease was transmitted from infected adults of *Eutettix tenella* feeding on the outer leaves of a plant to non-infected individuals on the inner leaves of the plant in 6 days. When the positions of the leaf-hoppers were reversed, the movement outwards took 10 days, at a slightly lower temperature.

The infective agent of curly-top was transmitted from infected hoppers on one of the first two leaves of a young beet plant to non-infected males on the opposite outer leaf at the end of 2 days at 81° F.

McCubbin and Smith (11) inoculated the main stem of tomato plants, the lower branches of which had been "layered" so as to induce rooting. By separating the daughter-plants after various intervals of time, they found that the virus agent travelled from 8 to 18 inches in 10–20 days. The virus agent apparently left the main plant between the 3rd and the 10th days.

Priode (12) found that, in the ringspot disease of tobacco, lesions frequently appear in the region of the inoculated leaf. Usually systemic infection follows. Holmes, in two papers (8, 9), has shown that the virus of tobacco mosaic may be found subsequent to inoculation above the inoculated leaf before it appears below. He has found that inoculation through broken trichomes takes place instantaneously, and that immediate washing off of the infected juice is not sufficient to prevent infection. Rubbing the infected juice on to wounds made some time before did not result in the appearance of symptoms.

Storey (17) found that the movement of the virus of the mosaic disease of maize across or down the leaf was not obviously impeded by cutting out portions of the midrib or by severing the veins of the half-lamina in which inoculation took place. "This result," he writes, "was to have been expected, for in either case the leaf remained turgid, being supplied by the small anastomosing veins which form a network through the leaf lamina." He found that movement down a leaf, subsequent to insect infection, was at a rate varying in six cases out of sixteen from 10 cm. to 20 cm. per hour.

Böning (4) found that the virus of mosaic disease travelled in tobacco leaf a distance of 13 cm. in a minimal period of 2 days and in tomato leaf a distance of 9 cm. in 2 days. In the tomato leaf "streak" travelled a shorter distance in 4 days. His results for rate of movement up and down the stem may be summarised in the following way. The results refer to tomato stems.

Table I.

Movement of virus agents in tomato stem (from Böning).

Mosaic.			*Streak.*		
Direction	Distance (cm.)	Time (days)	Direction	Distance (cm.)	Time (days)
Down stem	12	4	Down stem	12	6–7
Down stem	20	4–5	Down stem	20	4–5
Up stem	12	3–4	Up stem	12	4–5
Up stem	25	4–5	Up stem	25	5

It will be seen that there is some variation in the results, but they all do definitely suggest that the movement of the virus is comparatively slow, and that it moves in either direction in the plant at rates of the same order of magnitude.

Recently, Davis (7) has summarised the literature on the Infectious Chlorosis of Variegated Plants. He deals especially with the work of Baur and of Lindemuth. Baur found that the causative agent of chlorosis in *Abutilon avicennia* apparently moves through the extra cambial tissues. He prevented the movement by "ringing" the stem. He also suggested that the agent could move through an immune *A. arboreum* stem grafted on an *A. avicennia* without multiplication—the former stem being a mechanical means of transport. On the other hand, Blakeslee (3) has shown that the agent of the "Q" disease of Datura did not travel through a Petunia stock on an infected scion.

The problem here treated.

The evidence adduced from the experiments on the rate of movement gives one but little indication of the tissues in which the movement is taking place. It was proposed, therefore, to deal with that aspect of the problem.

Various general considerations operated in suggesting the lines along which work might be carried out. It has already been shown by various authors (Auchter (1), Curtis (6), Caldwell (5)) that, when substances moved in the phloem or in the xylem either upwards or downwards in the plant, movement was considerably more free in a vertical than in a lateral direction. The movement laterally was much less rapid, if, indeed, in some cases it took place at all.

The first experiments were, therefore, set up with a view to studying localisation of the movement of the virus in the tissues of the plant. When an eosin solution was absorbed by a petiole stump, subsequent to the removal of the lamina, the eosin was found to travel upwards and downwards on the same side, and to pass over to the other side of the plant only after it had travelled out to and round the anastomosing vessels at the leaf-tips. In plants with decussate phyllotaxis the localisation was particularly well marked (see Caldwell (5)). A simple analogy suggested itself with the substitution of virus inoculum for the eosin solution. All the experiments hereafter described were carried out using the "aucuba" disease of tomatoes—a mosaic disease which has been described in detail by Henderson Smith (15). This disease was chosen for the work in this investigation because it is easily transmitted by juice inoculation, is very infectious, and shows particularly well-defined symptoms. The symptom-complex has already been discussed by Henderson Smith, who described the main features, in bright weather under normal conditions, as follows: "Scattered over the leaf are patches of white and patches of yellow, usually sharply delineated but sometimes shading into neighbouring areas, irregular in shape and size, often angular, and occurring in all parts of the leaf." This disease is so infectious that it is easily transmitted merely by rubbing a healthy plant after infected plants have been handled. This feature is especially valuable when dealing with experiments yielding negative results, as, in the main, symptoms regularly follow inoculation.

Inoculations were made as follows: leaves from a plant showing aucuba symptoms were cut into small pieces and crushed in a mortar. Thereafter was added a volume of distilled water equivalent to twice the

weight of the leaf material. The whole was carefully pulped and mixed. A few drops of this material were transferred by means of a Pasteur pipette to the back of a marking label. This was held under the pinna to be inoculated, and another drop was placed on the upper surface. This drop was carefully spread over the whole of the upper side of the leaf. Some 30–40 holes were then pricked in the lamina—care being taken to distribute them as evenly as possible. In all cases where the plant as a whole was to be infected, four pinnae were treated, but in some cases—as when unilateral infection was attempted, only two pinnae (both on the same leaf) were so treated. All the apparatus used was sterilised so far as was possible, and the whole operation was carried out with the minimal chances of accidental infection.

INOCULATION ON ONE SIDE.

It was thought from analogy with the movement of metabolites that, if the movement of the virus agent was localised in the vascular elements, the tendency would be for symptoms to appear in the region of the treated shoot or leaf. Especially should the leaves on the same orthostichy show symptoms rapidly. As has before been pointed out, the axillary shoot has similar vascular connections to the leaf in the axil of which it develops. In the main, symptoms appear first on the younger leaves which are, naturally, at the top of the plant. As the axillary buds develop, however, their leaves also show symptoms.

In the first experimental plant C_2 inoculation was made on July 8th on the leaves of a single branch. Symptoms appeared at the top of the plant and on the leaves of the axillary bud above the treated shoot on July 21st. Two other shoots on the same side showed symptoms by July 22nd. The symptoms appeared on the leaves of the opposite axillary shoots on July 26th. There was in this case, therefore, some delay in the appearance of the symptoms on the side away from the inoculation.

In the other plants, C_1, C_3, inoculated at the same time and on leaves of a single branch, the symptoms appeared first at the top and then downwards as the axillary bud-leaves grew without any apparent localisation. Similar results were obtained with a fourth plant, C_9, which was inoculated on July 18th. Four plants, A_5–A_8, inoculated on July 18th and on a single leaf all showed the symptoms systemically. Among four other plants, R_9–R_{12}, inoculated similarly on July 24th, three showed systemic infection, and in one there was a slight delay on the side away from the inoculation. Four large plants similarly treated by Dr Henderson Smith in October showed symptoms quite systemically. Out of

sixteen plants, therefore, one showed a delay of 4 days in the appearance of the symptoms on the further side as compared with the nearer, and one case had a doubtful delay of 1–2 days. The others were characterised by systemic infection. When it is borne in mind that the time elapsing before the appearance of symptoms is at all times rather variable, and may vary from 4–18 days under not dissimilar circumstances, the slight delay found in these cases may hardly be considered as significant.

Having been unable to demonstrate definitely the localisation of the movement of the virus to the side on which inoculation was made, another type of experiment was set up.

Effect of "ringing" the stem.

The results obtained by Baur (Davis (7)) and by Bennett (2) suggested that the effect of "ringing" was to stop the movement of the infective agent. This did not appear to be so in the case of tomato, as in all of the four tomato plants tried symptoms did appear on the parts of the plants on the non-inoculated side of the "ring." In this plant regeneration takes place very quickly—the stelar tissue is essentially parenchymatous in nature. (There is further a feebly developed intraxylary phloem in the tomato.) As a consequence of this it was difficult to decide whether the symptoms appeared as a result of infection by a virus which had moved very slowly through the living cells in the xylem and the pith, or else had travelled through the phloem tissue which arose as a result of the regeneration of the tissues.

Experiments with partially killed stems.

As it was not possible to rely on the non-recovery of the "ringed" stem, an attempt was made to remove the living cells in the stem by killing them. The agent used was chloroform, which destroys the protoplasm of the parenchymatous cells and which has, apparently, but little effect on the virus of aucuba mosaic (see Henderson Smith (15)).

A region in an internode about the middle of a fairly large stem some 40 cm. high was killed over a distance of 5 cm. This was done by making two incisions at right angles through the stem with a sharp scalpel. The incisions were 1–2 cm. long. Into them was put chloroform which was also applied to the outside of the stem. The plant was first staked up and lightly tied to the stake. After some hours the outside of the treated portion of the stem was smeared with vaseline. The effect of the treatment with chloroform was seen rapidly. The tissues almost immediately lost their turgidity and at first looked as if they were waterlogged. By the

next day the treated area and a portion on either side of it had become brown and quite dead. On that day the plants were inoculated either above or below the lesion. In other cases the stem treated was that of a branch, and the distal end of the branch was treated as being "above" the lesion.

In these plants which had been so treated, the effect of the killing of the living tissue of the stem early became apparent. Adventitious roots appeared above the lesion, especially on the region immediately above the killed tissues. The appearance of one such plant is illustrated in Plate XXXIII, fig. 1. The results are given below in tabular form. Four experiments were carried out with branches treated and twenty-two with stems. In some cases the inoculation was made "above" the lesion, and in others "below." The plants were all inoculated between July 1st and July 24th, 1929.

Table II.

Details of plants inoculated after the stem had been treated with chloroform.

Tota number of treated plants (July 1st–24th, 1929)	26
No. of plants when infection did not pass lesion	14
No. of plants when infection was not delayed by lesion	4
No. of plants where tissues partially recovered	4
No. of plants where symptoms appeared across lesion	4

It can be seen in this table that fourteen plants showed no symptoms on the opposite side of the lesion. In twelve plants symptoms did appear across the lesion. There is good evidence to account for this result in the case of eight of the plants. In the four plants where "the infection was not delayed by lesion" the symptoms appeared simultaneously on either side of the lesion. It was, therefore, presumed that the "killing" of the tissues had not been quite complete and that some living cells had remained. In the four plants of the next section there was regeneration of the tissues secondarily, so that long after the symptoms had appeared on one side of the "killed" tissue the infective agent was able to move across the regenerated tissue, and to cause the appearance of symptoms on the opposite side. In the last four plants the symptoms appeared after a delay of 4–5 days and the tissue had not regenerated. It is suggested that these plants were probably accidentally infected by handling.

The whole experiment was repeated on August 23rd and 28th and September 4th–5th, 1929. The plants in this case were not now treated with chloroform, but the stems were treated with steam in a fashion similar to the chloroform treatment. A piece of damp cotton-wool was

wrapped round the stem at the middle of a suitable internode. This cotton-wool in turn was held in place by a small sheet of tin-foil wrapped round it. The outside of the tin-foil was heated with a small gas jet and the water on the cotton-wool boiled. Care was taken to protect the leaves above and below the treated internode. After this treatment the plants were left for one day, after which the outside of the dead area was vaselined. The stems in these plants again were tied to canes to prevent their falling. Inoculation was made below the killed tissues in each case.

In the first three plants, *S* 21–*S* 23, symptoms appeared below the lesions in 5 days. In *S* 24–*S* 26 they appeared in 8 days. In the plants *S* 30–*S* 44 the symptoms appeared between the 5th and the 7th day after inoculation. Untreated controls showed symptoms on the 5th day. Only one out of these plants showed symptoms above the killed area.

Table III.

Data relating to the plants with "steamed" stem.

Total number of plants	21
No. of plants showing no symptom above lesion	20
No. of plants showing symptoms above lesion	1

The plants which were used for the experiments had, unfortunately, to be tested for latent virus by being kept for 4 weeks or so. It was not possible to keep the portions above the lesions turgid for as long a time, and usually the leaves tended to wilt about the 10th day after treatment. For this reason, it was necessary to remove the upper portion of the plant just above the killed area at the end of a fortnight and to grow it separately as a cutting. The cuttings were then examined daily for symptoms.

That the xylem water current had not been cut off by the treatment received was indicated by one or two observations. (*a*) The upper portion of the treated plants remained quite turgid for over a week, usually for a fortnight. (*b*) The xylem vessels appeared quite open on sectioning the tissue. (*c*) The shoots enlongated after treatment and appeared quite normal. In the case of one of the treated branches measurements were made on two of the leaves distal to the killed tissue. The figures are given below:

Table IV.

Leaf	Length on July 3rd (mm.)	Length on July 10th (mm.)
A	22	37
B	35	60

It will be seen that in one week the increase was nearly 100 per cent. Another and probably more satisfactory method of determining if water were passing up the stem across the dead area was the following. The stem of a treated plant was removed just above the roots and the cut end immersed in a solution of eosin, or some other dye. The solution travelled up the stem and passed across the treated area without obvious delay. Similar results were obtained when the dye was absorbed at the cut end of a petiole either above or below the lesion. The solution passed from below upwards or from above downwards quite readily—the dead tissues offering no apparent obstacle to movement. The movement of Chinese ink has taken place up to 3 weeks after the killing of the tissues.

MOVEMENT OF PARTICULATE SUBSTANCES THROUGH KILLED STEMS.

Of special interest in this connection are the experiments with diluted Chinese ink and nigrosin in water. When the cut stem was placed in either of these substances, the movement through the killed area of the stem could readily be watched by noting the blackening of the vascular bundles. The movement of these materials was not stopped at the top of the lesion but, on sectioning, particles of them were found in the vascular elements of the distal portion. This proves that the vessels were not plugged with protein materials, which might have acted as colloidal filters preventing the free movement of a virus body while allowing the possibly smaller eosin molecules to pass across.

Snow (16) has found that very similar treatment in the case of bean seedlings killed the living tissues but did not, at first, impede the movement of water. He found that the distal portion of plants in which an area of stem had been killed remained turgid for some 3 weeks.

The upper portion of the plant rooted readily as a cutting, and showed symptoms comparatively rapidly after being established if it had been previously infected. Those from treated stems were grown as plants for 4 weeks before being considered as clean. The cutting was always taken just above the dead tissue.

To ensure that the time (14 days) which elapsed between inoculation and wilting was sufficient to admit of the movement of virus up a normal stem, various untreated plants were inoculated. In some of these cases the minimal dose was given—inoculations being made on a single pinna at the base of the plant. After 4, 5 or 6 days the tops were removed some five or six internodes above the inoculation. Only one of the 4-day plants did not show symptoms on the upper portion which had been treated as a cutting.

Further, controls of the same size as the treated plants, inoculated at the same time but without steamed tissue, were arranged. In every case symptoms appeared, at the top of the controls, before the distal portion of the treated plants had wilted.

The mere fact of using the tops as cuttings to test if the virus were present in them is not in itself sufficient to inhibit the development of symptoms in infected material. It has been pointed out that infected stems will root and show symptoms comparatively rapidly. If young leaves are allowed to root on sand, it has been found by the writer that subsequent inoculation on one pinna results in the appearance of virus symptoms over the whole leaf. Purdy (13) found similar results with tobacco leaves. To ensure, further, that no top was a "carrier," the leaves of some of the tops were removed at different stages and inoculated, as above described, into batches of four young actively growing plants. In no instance did the test plants develop symptoms. Incidentally, no case of a "carrier" for aucuba mosaic has yet appeared in our cultures.

To determine if any virus had passed into the dead tissue and had there been adsorbed, the following inoculations were made. The region of dead tissue was divided into three so that the middle portion was quite free from the living tissue above or below. The three portions were approximately equal. Inoculations of each of these three portions were made into sets of plants. In the first case, the lower third, all the plants developed symptoms, but in the other two sets all the plants were clean.

Possible adsorption of the virus agent.

One possibility suggested itself in the matter of the non-crossing of the killed area. It was thought that the actual killing of the tissues might have liberated some substance which inhibited the development of symptoms above the lesion, or, alternatively, the dead tissue might have absorbed the virus agent and prevented its further passage. To ascertain if either of these suggestions were probable, the following set of experiments was carried out.

A quantity of macerated leaf-tissue from virus plants was divided into three portions. One portion was inoculated directly into the first set of plants. These served as controls. The second portion was centrifuged at a high speed for 5 minutes; then thoroughly shaken up; again centrifuged for 10 minutes and thoroughly shaken. After having been centrifuged for another 10-minute period the supernatant liquid was decanted. The residue was mixed with a little sterilised distilled water. The super-

natant liquid was a clear brown colour. Sets of plants were inoculated with the supernatant liquid and with the residual material.

The third portion of the infective material was passed through muslin to remove the larger pieces of tissue. To the filtrate was added a quantity of stem tissue of uninfected plants which had been steamed in the manner previously described. The whole was carefully mixed together. The mixture was treated in exactly the same way as was the green tissue. It was alternately mixed and centrifuged. Thereafter the supernatant and the residual material were inoculated separately into sets of plants. There were, therefore, five sets of plants, viz.

(*a*) Controls inoculated with untreated macerated material.

(*b*) Plants inoculated with supernatant liquid of centrifuged macerated material.

(*c*) Plants inoculated with residue of centrifuged macerated material.

(*d*) Plants inoculated with supernatant liquid of centrifuged mixture of infected juice and boiled tissue.

(*e*) Plants inoculated with residue of centrifuged mixture of infected juice and boiled tissue.

These plants were set up on October 16th, 1929. Most of them showed symptoms of aucuba mosaic by October 29th. All were definitely infected when they were discarded on November 29th. The effect of the boiled tissue, therefore, was not appreciable under the conditions obtaining in these experiments.

DISCUSSION.

From the results of the experiments detailed above, it is evident that one point has been established regarding the movement of the virus of aucuba mosaic in tomato. It has been clearly demonstrated that the virus agent did not travel across tissue of which the living elements have been killed. Through this tissue, on the other hand, water could and did pass. The xylem vessels were not blocked, and coloured solutions passed freely upwards or downwards. No adsorption or inhibition of the causative agent could be demonstrated. One is, therefore, forced to the conclusion that the virus agent cannot travel mechanically in the xylem stream, but can only pass through the living tissue. For some reason, as yet not understood, survival of the tissue is necessary for the movement of the virus (cf. Holmes (9)). This is of more than passing interest, when it is remembered that the virus of tobacco, for example, can withstand the curing processes. In my own experiments, I have found that the

dead, dry leaves of aucuba infected plants continued to be infective when inoculated into fresh plants.

The conclusion is in complete agreement with that of Baur and of Bennett. It does not confirm the implied suggestion of Storey, who pointed out that it was not surprising that the virus travelled across a cut lamina, since the opposite side was still turgid. The implication is that, if the water passed across the leaf, the virus should also have passed. This is not necessarily so. Auchter has demonstrated, and I have confirmed his results, that there is distinct localisation in the movement of salts, etc., in the plant. It appears that, in the main, rapid lateral movement in plant tissues is confined almost entirely to water; even water-soluble salts do not appear to travel so rapidly.

It may, therefore, be stated definitely that, for the virus principles which have been studied, there is no direct evidence for movement in the xylem. All the experiments have, so far, shown that movement does not take place through the xylem tissues.

Movement in the phloem.

Movement through the phloem is more difficult of demonstration. The evidence adduced on this point must, in the nature of things, be circumstantial. It is not practicable to isolate "phloem" from "living" tissues. Two lines of argument may be pursued. If the movement were necessarily confined to the phloem tissues, there would be, it is suggested, some evidence of localisation of the symptoms to one particular portion of the plant. The leaves on the axillary shoot of the inoculated leaf and those directly above and below would, presumably, show symptoms first. The leaves on the alternate sides, that is, those with petioles more or less at right angles to the treated leaf, would next develop symptoms. The basis of this argument and the data concerned are contained in the papers on the movement of materials in plants (Caldwell (5)). The leaves on the side opposite to the treated leaf should not develop symptoms until long after the others. If movement were extremely rapid this might not necessarily hold. It has been shown, however, that it is not.

In the experiments recorded above, only one somewhat doubtful case occurred in which symptoms appeared some few days later on the opposite side. Even if this were a valid instance it is isolated, and, in any case, the difference in time is much too small to have any great value. The general conclusion to be derived from the experiments here recorded and from numerous others is that in the tomato, at least, there is no evidence whatsoever for localisation of movement to any sector of the plant. Severin

actually reports cases where the virus had, apparently, passed down the petiole of a leaf, had crossed the developing "bulb" of a beet, and passed up the petiole of the opposite leaf. The whole operation, including, presumably, some multiplication in the tissues of the opposite leaf, had occupied 2 days. This suggests, in the light of the work referred to above, that movement must have taken place across tissues other than phloem.

The rates at which movement takes place in the tissues are of value in this connection. Storey and Severin have measured movement within a petiole or within a leaf-blade. The data they report are given in the following table.

Table V.

Rate of movement of the virus of sugar beet and of maize.

Time (hr.)	Virus	Plant	Distance travelled (cm.)	Author
½	Curly-top	Beet	17·5	Severin
½	„	„	8·1	„
1	„	„	8·75 (twice)	„
1	„	„	11·8	„
1	„	„	13·1	„
1	Mosaic	Maize	10 (thrice)	Storey
2	„	„	40 „	„

In Table I Böning's data for tomato mosaic and for "streak" have already been given. In my experiments rates of the same order of magnitude were obtained, though there is always some variation due to individual differences in the plants used. There is no reason to assume from these data that the agent was moving in the xylem: rather there is strong evidence that it was not. It is difficult to believe that the infective principle could move so slowly in the water stream. Most experiments suggest a rate of some 2 mm. per hour, following needle inoculation. Any data available suggest a very much greater rate of movement for water in the xylem tissues (cf. Sachs, etc.).

Movement in the leaf tissues appears to be very much faster. In this case the material used was inoculated into the tissues by insect-vectors. These insects are known to penetrate by means of their stylets right to the phloem tissues. It is, therefore, suggested that the few observations which give very quick rates of movement were made when fortuitously the infective insect had inoculated its virus right into the phloem of one of the larger veins and when the second insect had sucked the infective juice out of the same vein. In the majority of cases, however, movement takes place from cell to cell by some mechanism of diffusion. In cases

where the phloem is injected and not seriously upset movement is more rapid (cf. Mason and Maskell (10)). This probably accounts for the isolated cases which periodically occur in work with the tomato, where symptoms appear within 4 days of inoculation. As has before been noted the symptoms first appear in the rapidly developing apical leaves.

The following general conclusion must, therefore, be drawn as a result of a consideration of the work above, viz. that there is no evidence of movement in the xylem. Direct evidence indicates that movement does not occur there in the case of, at least, three types of virus agent. Bennett has found that the curl virus of raspberry will not travel across ringed stems. Baur obtained similar results with the infectious chlorosis of *Abutilon*. I have, in this paper, shown that the causative agent of *aucuba* mosaic of tomato does not cross regions of stem where the living tissues have been killed.

As for the extra-cambial tissues, the absence of any apparent difficulty of lateral movement of the virus agent in the plants points to there being no inability on the part of the virus to travel in any living tissue. Direct evidence from inoculation through trichomes supports this view. On the other hand movement may, on occasion, be so rapid as to indicate that it must have taken place in the phloem elements.

Summary.

In this paper the movement in the plant of the causative agent of virus disease is discussed. The relevant data in the literature are summarised.

A method is described whereby a portion of the stem in the middle of a tomato plant was killed either by chloroform or by steam. In this way the living upper and lower portions of the plant were connected by a bridge of dead tissue. It is shown that the symptoms appeared in that part of the plant in which the inoculation was made. The virus agent did not travel across the dead region.

The xylem tracts were not materially affected by this treatment, and water travelled across the region. Evidence of this is the fact that the distal portion remained turgid and sometimes continued growth for a considerable time. If the stem were removed above the ground level and put into eosin solution, this travelled readily over the dead tissue. That the vessels were not occluded by protein plugs is shown by the fact that particulate substances were carried up the xylem tracts past the dead region.

No evidence of adsorption of the virus agent to the cell remains could be adduced, so it is assumed that it was not travelling in the xylem stream.

CALDWELL.—THE PHYSIOLOGY OF VIRUS DISEASES IN PLANTS (pp. 429–443).

From previous experiments, it is known that the movement of metabolites and of stains tends to be localised to the side of the plant into which they are introduced. It was found that inoculation with juice of diseased plants caused systemic infection in all the treated plants. There was no apparent localisation of movement such as would have been expected had it been taking place through the vascular system. From this, and from other evidence in the literature, it is concluded that movement takes place in the living ground tissue of the plant.

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EXPLANATION OF PLATE XXXIII

In this plant the stem at one internode had been "steamed." Subsequent to inoculation below symptoms appeared on the lower part of the plant while the upper part remained healthy.

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THE RÔLE OF *THRIPS TABACI* LINDEMAN IN THE TRANSMISSION OF VIRUS DISEASES OF TOMATO

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Introduction.

The rôle of insects in the transmission of virus diseases of plants has long been recognised, but there is still uncertainty concerning the vectors of the virus diseases of tomato. White fly, *Trialeurodes vaporariorum* West, does not appear to transmit these diseases, Gardner and Kendrick (3) regard plant lice and flea beetles as possible carriers, while Olitsky (8) gives evidence of transmission of tomato mosaic by the "mealy" bug, *Pseudococcus citri*. Vanterpool (12) was able to demonstrate the transmission of tomato streak by aphides, but the latter writer does not give any details of the experiments or the insects used.

Many of the insects transmitting virus diseases belong to the order *Homoptera*, and possessing sucking mouth-parts to penetrate the plant tissues and feed on its juices, they would appear to be natural vectors of virus diseases. The possession of similar mouth-parts by members of the order *Thysanoptera*, and their prevalence both in the field and in glasshouses, has directed suspicion to these insects as possible vectors also. On account of their minute size and their habits they are difficult to control by insecticides and, therefore, are usually to be found in otherwise insect-proof glasshouses. In spite of these features they are not regarded as vectors by most workers and it was not until Pittman (9) obtained transmission of "spotted wilt" of tomatoes with *Thrips tabaci* Lindeman in Australia that serious attention was attracted to them. Schaffnit (10) mentioned *Thrips tabaci* Lindeman as a possible vector of mosaic diseases of beet and spinach, and Böning (1) regarded *Thrips flavus* Schrank as a possible vector of bean mosaic, but neither case has been confirmed.

The experiments described in this paper were carried out with *Thrips tabaci* Lindeman and virus diseases affecting tomatoes.

MATERIAL AND METHODS.

(1) *Source of insects.*

These were obtained from a commercial glasshouse where they were found in large numbers on virus-free cucumber plants. Subsequently they were cultured on healthy tomato plants.

(2) *Source of plants.*

The tomato plants of Kondine Red variety used in these experiments were grown from seed under conditions as nearly insect free as is possible in an insect-proof glasshouse which was fumigated regularly. The plants were young and rapidly growing at the time when the infected thrips were placed on them.

(3) *Source of viruses.*

The viruses employed were from various sources, and each had been filtered through Pasteur-Chamberland L. 1 and L. 3 candles previously and artificially inoculated with a needle into successive series of tomato plants.

(i) *Tobacco mosaic* (= *Tobacco virus* 1 (7)). This came from Dr Grainger of Leeds University who obtained it from Dr Johnston of Wisconsin originally.

(ii) *Glasshouse streak.* The source of this inoculum was a commercial glasshouse. The plants showed the irregular, dark, necrotic lesions on the stems, petioles and leaves characteristic of the disease streak or stripe. In addition, the younger leaves of the plants showed the coarse mottle or mosaic usually associated with necrotic symptoms in streak. The virus producing this mosaic has been shown (6) to be indistinguishable from tobacco virus.

(iii) *Potato mosaic.* The virus of potato mosaic which produces regular, necrotic spotting on the leaves of tomatoes, as described by Henderson Smith (5), was used in combination with (*a*) tobacco virus 1, and (*b*) the filtered extract of plants inoculated with glasshouse streak but showing the mosaic only. Both combinations produce a severe disease which is here termed experimental streak, in order to distinguish it from glasshouse streak, from which it differs mainly in the regularity and ease with which it can be transmitted by needle inoculation.

(4) *Methods.*

Young tomato plants growing in 6-inch pots were artificially inoculated with a needle about 10 days before each experiment, in order to provide a source of infection from which to infect the insects. A hurricane

lamp-glass chimney was placed over each plant, the base being firmly implanted in the soil, and the top covered securely with a piece of fine silk, thus forming an insect-proof chamber. Each plant was kept in its chamber while the thrips were colonised on it.

In each experiment, a certain number of insects were placed on infected plants and left to feed for varying periods of time, and then the insects were transferred to healthy plants in similar insect-proof chambers. This transfer had to be effected with great care, and to prevent any infected juice from broken hairs, etc. being carried over by the brush, with the insect, to the healthy plant. In order to eliminate this mechanical means of infection, the insects were taken off the infected plants with one camel-hair brush, placed in a petri dish, which was lined with black blotting paper so that the insects could be seen clearly and, with another clean brush, the desired number of infected insects were transferred to the healthy plants. After the insects had fed for the required time, the lamp glasses were taken off and the insects carefully removed from each plant, which was also sprayed with nicotine and soft soap.

In order to prove that the insects themselves were not carrying any disease from their source, suitable controls were set up with healthy tomato plants. In the second and third experiments, controls were also made to test the second series of brushes used in transferring the infected insects to the healthy plants. This was done by rubbing each brush over the young leaves of healthy tomato plants, different brushes having been used for each virus.

Details of experiments.

I. 7. v. 29. Fifty imagos and 50 nymphs were colonised on each infected plant for 7 days, and then varying numbers were transferred to healthy plants for a similar period.

	No. of plants		No. of infected thrips on each healthy plant		
Inoculum	Infected	Healthy	Imagos	Nymphs	Infection
Tobacco mosaic	2	2	(1) 16 (2) —	— 11	Nil
Glasshouse streak	2	2	(1) 17 (2) —	— 6	,,
Glasshouse streak showing mosaic symptoms only	2	3	(1) 15 (2) 12 (3) —	— — 13	,,
Experimental streak, No. 2	2	2	(1) 13 (2) —	— 4	,,

II. 16. v. 29. Two hundred imagos were colonised on each infected plant for 6 days, and then varying numbers were transferred to healthy plants for a similar length of time.

Inoculum	No. of plants		No. of infected thrips on each healthy plant	Infection
	Infected	Healthy		
Tobacco mosaic	2	2	(1) 25 (2) 16	Nil
Glasshouse streak	2	2	(1) 25 (2) 15	„
Glasshouse streak showing mosaic symptoms only	2	2	(1) 25 (2) 19	„
Experimental streak, No. 1	3	2	(1) 25 (2) 12	„
Experimental streak, No. 2	4	3	(1) 25 (2) 15 (3) 9	„

III. 23. v. 29. Two hundred imagos were colonised on each infected plant for 5 days, and then a definite number were transferred to each healthy plant for 9 days.

Inoculum	No. of plants		No. of thrips on each healthy plant	Infection
	Infected	Healthy		
Tobacco mosaic	2	3	(1) 35 (2) 25 (3) 15	Nil
Glasshouse streak	2	3	(1) 35 (2) 25 (3) 15	„
Experimental streak, No. 1	4	3	(1) 35 (2) 25 (3) 15	„
Experimental streak, No. 2	4	3	(1) 35 (2) 25 (3) 15	„

Experimental streak, No. 1, was produced by combining the virus of potato mosaic in tomato, and tobacco virus 1.

Experimental streak, No. 2, was produced by combining the virus of potato mosaic in tomato, with the filtered extract of plants inoculated with glasshouse streak, but showing the mosaic only.

The number of healthy plants colonised with infected insects was low in each series, on account of the high mortality after the insects were transferred from cucumber to diseased tomato plants. The large area of necrotic tissue on the leaves of plants inoculated with forms of streak probably accounted for the death of the less active insects placed on these

plants. The mortality of the infected thrips after their transference to healthy tomatoes was low.

That the insects had fed readily on both the diseased and the healthy plants was shown by the number of white areas on the leaves where the epidermis had been punctured.

Summary of insect controls.

Control	No. of plants	No. of thrips per plant		No. of days on each plant	Infection
		Imagos	Nymphs		
Experiment I	5	50	50	14	Nil
Experiment II	5	100	—	12	,,
Experiment III	5	200	—	14	,,

In each of the above controls, the insects were colonised on young healthy plants showing three or four leaves, and enclosed under lamp glasses for a period of time equal to the duration of the corresponding experiment.

After the insects of each series in Exps. II and III were transferred from infected to healthy plants, the second brush used in the transfer was firmly rubbed over the young leaves of two healthy tomato plants. None of these plants developed any signs of disease, therefore we may conclude that no infection was carried on these brushes.

In the following table, the numbers of healthy plants used in the above experiments are grouped together according to the inoculum from which the infected insects colonised on them were derived.

Experiment	Tobacco mosaic	Glasshouse streak showing		Experimental streak	
		Streak and mosaic	Mosaic only	No. 1	No. 2
No. I	2	2	3	—	2
No. II	2	2	2	2	3
No. III	3	3	—	3	3
Total	7	7	5	5	8

Thus seven plants were colonised with thrips from plants inoculated with tobacco mosaic alone, and five from plants inoculated with tobacco mosaic combined with potato mosaic (= experimental streak, No. 1). In none of these cases was the virus transmitted, so that there are twelve cases of failure to transmit tobacco mosaic.

Seven plants were colonised with thrips from plants inoculated with glasshouse streak showing both typical necrosis and the mosaic, five with

thrips from plants inoculated with glasshouse streak but showing the mosaic only, and eight with thrips from plants inoculated with the latter combined with potato mosaic (= experimental streak, No. 2). Again there was no transmission, that is there were twenty cases of failure to transmit the virus of glasshouse streak.

If streak is a severe form of tobacco mosaic in tomatoes (6), then there are, in all, 32 (12 and 20) cases of failure to transmit tobacco virus 1.

Five plants were colonised with thrips from plants inoculated with potato mosaic combined with tobacco mosaic (= experimental streak, No. 1), and eight with thrips from plants inoculated with potato mosaic combined with the extract of plants inoculated with glasshouse streak but showing the mosaic only (= experimental streak, No. 2). As there was no case of transmission, there are 13 failures to transmit potato mosaic in tomatoes.

DISCUSSION.

The failure of the above experiments to demonstrate transmission of these viruses is somewhat surprising, when one considers that Pittman readily obtained transmission of "spotted wilt" of tomatoes in Australia, using only three to five nymphs per plant.

It is, of course, possible that the Australian disease, "Spotted Wilt," is not identically the same disease as English streak, for neither Brittlebank (2), Hamblin (4) nor Pittman (9) has succeeded in transmitting "spotted wilt" by artificial inoculation of extracts from diseased into healthy plants. On the other hand, Brittlebank (2) concludes that "spotted wilt" in Australia is probably identical with winter blight of tomatoes in America, as described by Selby (11), and Vanterpool also regards these diseases as identical. The American disease corresponds in all particulars to the streak or stripe found in England. From personal observation of "spotted wilt" in Australia, the writer regards the disease as indistinguishable from the English disease. The comparison of these diseases is made purely on symptoms, and it is recognised that symptoms alone are not a safe basis for identification, but as yet no other criteria are available in the case of "spotted wilt."

Again, it is possible that the insect, *Thrips tabaci* Lindeman, used by Pittman was of different strain and habits to the English insect of the same identification used in these experiments. Moreover, the conditions such as light, temperature and humidity under which these experiments were conducted may not have been favourable to transmission of the disease by the thrips, although they feed freely on the plants. It is clear

that *Thrips tabaci* does not transmit virus diseases of tomato under all conditions.

However, one positive result such as that of Pittman is of more value than many negative ones, and if it cannot be repeated, either the conditions or the materials with which it was obtained have not been completely reproduced, or it is due to unnoticed factors which can only be detected in repetition of experiments.

Summary.

A description is given of experiments designed to show the rôle of *Thrips tabaci* Lindeman in the transmission of tomatoes.

The diseases tested were tobacco mosaic and glasshouse streak singly, and the viruses of each of these two combined with a potato mosaic virus to give a disease termed experimental streak.

The source of the materials used and the methods employed are described in detail.

In no case was transmission of any of the viruses recorded, although the insects had fed freely on all the plants. It is concluded that *Thrips tabaci* does not transmit virus diseases of tomatoes under all conditions. The importance of this insect as a vector of these diseases in commercial glasshouses in England is therefore doubtful.

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A STUDY OF THE DEGENERATION OF CERTAIN POTATO STOCKS

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(With Plates XXXIV and XXXV, 8 Graphs and 1 Text-figure.)

CONTENTS.

I. Introduction.

Although it is now usually accepted that degeneration in potatoes is largely—if not entirely—due to the accumulation of virus-infected plants in the crop, there has been no critical work published, so far as the writer is aware, which shows the *degree* of relationship between the progressive reduction in yield of potato stocks and the incidence of infection

with virus diseases. This relation can be studied either by proving the absence of degeneration so long as infection is excluded from the crop, or by determining the correlation between loss in yield and the amount of virus disease when stocks are allowed to degenerate "naturally." The former method cannot properly be used until it is shown that perfectly healthy crops can be produced and maintained for a number of years. The latter method, however, is comparatively straightforward, and has been used by the writer with stocks of the varieties Kerr's Pink and Great Scot maintained by the Department of Agriculture of this College for periods varying from one to seven years without change of seed. The incidence of virus diseases and the cropping power were studied each year and, finally, chequerboard trials were laid down in 1926 in the case of the Kerr's Pink, and in 1927 with Great Scot. It is the main purpose of this paper to discuss the factors affecting the rate of degeneration in these stocks of potatoes.

II. Chequerboard trial with Kerr's Pink.

In 1926 there were available for planting, once, twice, thrice, etc., up to six times grown seed, together with new Scotch seed from a reliable grower in Banffshire, obtained in the autumn of 1925 and boxed, so as to make them in every way comparable with the home-saved seed. The seed boxes were kept under close observation during the winter and, although no aphis infestation occurred in storage, the additional precaution was taken to fumigate at frequent intervals with nicotine dust. The stocks kept well and there was little loss from storage diseases. Before planting, all tubers were graded into ware, seed and chats, by passing them over $1\frac{3}{4}$ inch and $1\frac{1}{4}$ inch riddles, and then again sorted according to weight, so that the sets were finally grouped into ware = 3 to 4 tubers to the pound, seed = 6 to 8, and chats = 12 to 16 tubers to the pound. The tuber classes in any year class thus agreed, within fairly close limits, both as to size and weight, with those of the other year classes. The unit plot consisted of 56 tubers planted 15 inches apart in two rows of 28 tubers each. Seven replications of unit plots in each tuber and year class were "randomised" so that each year class occurred once, and only once, in either a north-south belt of plots, or east-west belt; the object of course being to minimise as far as possible any effect of soil variation on the yield, or on foliage symptoms of virus diseases. The whole trial was surrounded by a crop of new Scotch Kerr's Pink in order to eliminate marginal effects. The plots were planted on May 3rd–5th, and were lifted on October 18th–23rd. Rain fell almost continuously during

the lifting period and each east-west belt was raised in turn, the weights being taken only after the tubers were dried and the soil removed in the process of "sorting."

(*a*) *Variation in soil fertility.*

Although the ground occupied by the trial was as uniform as could be found, it sloped upwards from north to south, and it was necessary to see if any appreciable drift in fertility could be demonstrated. No such drift, however, could be detected in tables constructed to show the mean yields of all the plots in either the north-south belts or east-west belts. The trial ground was, therefore, regarded as sufficiently uniform for a statistical examination of the yields. The mean of the seven unit plots in each year or tuber class was regarded as representative of the cropping power of its class, the standard of accuracy being measured by the magnitude of the probable error of the mean yield, arrived at by the method of "least squares." These mean yields are given in Table I.

Table I.

Year class	Tuber class	Mean yield (lb.)	Mean no. misses	Yield corrected for misses (lb.)	P.E. of mean lb.	P.E. of mean %
1920	Ware	72·0	5·9	80·2	2·1	2·6
1921	,,	98·0	3·9	105·2	1·0	1·0
1922	,,	91·7	3·7	98·2	1·87	1·9
1923	,,	89·9	4·0	96·8	1·95	2·0
1924	,,	116·0	2·3	121·2	1·8	1·4
1925	,,	119·7	2·0	124·5	3·8	3·05
1926	,,	131·0	2·0	136·3	3·0	2·16
1920	Seed	59·4	6·4	66·8	1·37	2·05
1921	,,	85·1	4·1	91·6	1·81	1·97
1922	,,	82·6	5·6	91·6	2·32	2·53
1923	,,	80·1	3·6	85·4	1·22	1·44
1924	,,	101·1	3·0	107·0	3·05	2·85
1925	,,	102·3	1·7	105·7	3·52	3·33
1926	,,	116·4	2·1	121·4	3·6	2·9
1920	Chats	43·3	10·0	51·7	4·55	8·8
1921	,,	68·6	4·9	74·9	1·73	2·31
1922	,,	63·0	4·1	67·8	3·19	4·7
1923	,,	67·6	4·9	73·8	2·03	2·75
1924	,,	88·1	2·6	92·4	2·34	2·53
1925	,,	95·4	2·0	98·9	4·52	4·57
1926	,,	109·4	1·4	112·3	1·26	1·12

(b) *Correction for "misses."*

Although care was taken to remove from the seed boxes all tubers affected with sprout-destroying diseases, a number of tubers, when planted, failed to produce plants. These "misses" must in some way be allowed for in calculating yields, since their number varied from plot to plot. Tables II and III give the mean number of misses occurring in belts taken respectively from north to south and from east to west across the trial.

Table II.

Mean number of misses in each belt of plots from north to south.

Belt	1	2	3	4	5	6	7
Ware	4·7	3·4	3·7	3·9	2·4	2·7	3·3
Seed	2·7	2·0	4·1	4·9	3·9	4·7	4·3
Chats	2·7	2·9	5·6	4·7	4·7	5·3	3·0

Table III.

Mean number of misses in each belt of plots from east to west.

Belt	A	B	C	D	E	F	G
Ware	3·0	3·4	3·9	3·0	2·6	4·3	4·3
Seed	2·4	3·4	3·7	3·0	4·1	5·4	4·4
Chats	3·7	5·0	4·1	2·0	4·5	3·9	5·6

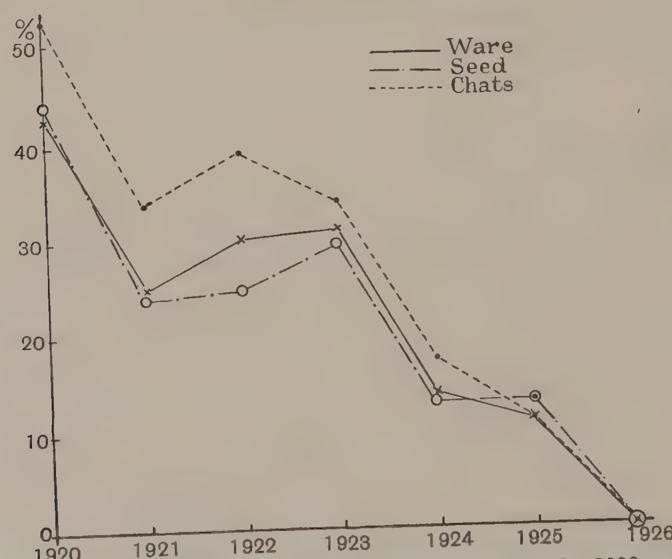
The distribution of the misses is apparently quite haphazard, and the number does not vary much in the three tuber classes. Reference, however, to Table I shows clearly that in all tuber classes the number of misses becomes progressively greater as the stocks get older. Possibly a few misses were due to rooks, but these would affect all years alike, so that the gradual increase of misses in the older stocks must be attributed to an intrinsic character of the tubers themselves. Any correction, therefore, must be based on the average yield of the remaining plants in the unit plot, for the potential value of a miss in a plot with a high average yield is greater than that of a miss in a plot giving only a low average yield per plant. Stewart (6) calculated that 50 per cent. of the loss due to a single miss was made good by the increased growth of the two neighbouring plants, but Salaman (4) found it necessary to assume that the adjoining plants were unable to benefit at all from the larger growing area due to the occurrence of a miss, and he corrected for such misses by adding to the total yield the average per plant for each miss. During the present work a note was taken of the position of each miss, but the data could not be used to compare the accuracy of the 50 and 100 per cent.

replacement methods, since very few unit plots had a complete stand of plants, and an entirely unknown factor was introduced by the variable number of virus-infected plants occurring in the different plots. The wholly arbitrary correction of allowing 100 per cent. replacement, therefore, has been followed in this trial.

Table I gives the mean yield of each set of seven unit plots corrected on this *pro rata* basis, together with the probable errors. It is to these corrected yields that reference is made in the following pages.

(c) *Discussion of yield results.*

Total yields. In comparing the yields from the various year classes, differences have only been regarded as significant when they exceed the probable error of the difference of the mean by at least three times. These differences in yield, in pounds, are given for the "ware" tuber class in Table IV, the years being arranged in order of decreasing yield



Graph 1. Percentage loss in yield of Kerr's Pink (new Scotch—1926—=0 %).

and the significant differences being shown in heavy type. Entirely similar tables have been constructed in the "seed" and "chat" tuber classes, but are omitted to save space.

It is clear that the stocks can be divided into a high yielding group (1926, 1925 and 1924) and a low yielding group consisting of the four older stocks, corresponding more or less with the agricultural practice

Table IV.

Kerr's Pink. Significance of difference in yield and virus infection with ware sets.

Leaf-roll (%)	Total virus disease (%)	Yield	Year	1926	1925	1924	1921	1922	1923	1920
					−11·8	−15·1	−31·1	−34·1	−39·5	−56·1
0·0	1·4	136·3	1926	—	−1·6	−6·8	−15·5	−31·2	−25·4	−50·4
					−2·5	−14·6	−33·1	−50·0	−50·6	−81·3
				+11·8		*−3·3*	−19·3	−26·3	−27·7	−44·3
1·6	3·9	124·5	1925	+1·6	—	−5·2	−13·9	−29·6	−23·8	−48·8
				+2·5		−10·1	−30·6	−47·5	−49·0	−78·8
				+15·1	*+3·3*		−16·0	−23·0	−24·4	−41·0
6·8	16·0	121·2	1924	+6·8	+5·2	—	−8·7	−24·4	−18·6	−43·6
				+14·6	+10·1		−18·5	−35·4	−36·9	−66·7
				+31·1	+19·3	+16·0		−7·0	−8·4	−25·0
15·5	34·5	105·2	1921	+15·5	+13·9	+8·7	—	−25·7	−9·9	−34·9
				+33·1	+30·6	+18·5		−17·9	−18·4	−48·2
				+34·1	+26·3	+23·0	+7·0		*−1·4*	−18·0
31·2	51·4	98·2	1922	+31·2	+29·6	+24·4	+25·7	—	*−5·8*	−19·2
				+50·0	+47·5	+35·4	+17·9		*−1·5*	−31·3
				+39·5	+27·7	+24·4	+8·4	*+1·4*		−16·6
25·4	52·0	96·8	1923	+25·4	+23·8	+18·6	+9·9	*+5·8*	—	−25·0
				+50·6	+49·0	+36·9	+18·4	*+1·5*		−29·8
				+56·1	+44·3	+41·0	+25·0	+18·0	+16·6	
50·4	82·7	80·2	1920	+50·4	+48·8	+43·6	+34·9	+19·2	+25·0	—
				+81·3	+78·8	+66·7	+48·2	+31·3	+29·8	

In each group, upper figure = difference in yield (lb.).
middle figure = difference in % leaf-roll.
bottom figure = difference in % total virus infection.

of changing the seed every two or three years. If the crop from the new Scotch (1926) tubers is represented by 100 and that from the other stocks expressed as percentages of this, the gradual deterioration is as shown in Table V.

Table V.

Percentage reduction in total yield in 1926 from the three tuber classes.

Tubers planted	Year in which stocks were obtained from Scotland						
	1926	1925	1924	1923	1922	1921	1920
Ware	0·0	8·7	11·1	29·0	28·0	22·8	41·2
Seed	0·0	12·9	11·9	29·6	24·6	24·6	45·0
Chats	0·0	11·9	17·7	34·3	39·6	33·3	54·0

In the younger stocks the deterioration does not appear to have been affected by the size of tuber planted, but in the older ones the chat sets have certainly produced a poorer crop relative to new Scotch chats than either the seed or ware sets. The same point is brought out in Graph 1.

The significance of these differences in yield can be summarised as follows:

(1) The yield from all the 1920 tuber classes was significantly less than that from any other stock.

(2) All tuber classes of 1921 stocks gave significantly higher yields than 1920, and definitely less yields than 1924, 1925 and 1926. The yield from planting seed or chats was as good as from the same tuber classes of the 1922 and 1923 stocks, whilst the ware sets gave a significantly *greater* yield than either 1922 or 1923. This relatively high yield of the 1921 tuber classes is of particular interest, and will be referred to later. It is, in itself, sufficient evidence that mere continued saving of home-grown seed is not a complete explanation of degeneration.

(3) The 1922 seed and chat sets produced as good a crop as that from the 1921 and 1923 stocks, but the ware was definitely inferior to the 1921 ware. All tuber classes were significantly poorer than 1924, 1925 and 1926, and better than 1920.

(4) The 1923 stocks were no better yielders than the 1921 or 1922 stocks when seed or chats were planted, and with ware a definitely smaller yield was obtained as compared with 1921. All stocks, however, were significantly better than 1920.

(5) All the tuber classes of the 1924 stocks gave as good a yield as the 1925 stocks. They cropped better than any of the older year classes but were significantly poorer than 1926.

(6) The 1925 stocks were no better than the 1924. When compared with 1926 there was no significant difference in yield when ware sets were used, but with seed-size sets the 1925 stocks were definitely inferior, and possibly also with chats since the difference in yield was very nearly three times (*i.e.* 2·85) the probable error.

(7) With the exception of the 1925 ware sets, and possibly also the 1925 chats, all the tuber classes of the new Scotch stocks were significantly better than any of the home-saved stocks.

(*d*) *Effect of "age" of stocks on the size of tubers produced.*

The percentage of "ware," *i.e.* tubers retained by a $1\frac{3}{4}$ inch mesh, produced when each of the three tuber classes was planted is given in Table VI.

Table VI.

Mean percentage of ware tubers in crop from each year and tuber class.

Year class	Ware planted	P.E.	Seed planted	P.E.	Chats planted	P.E.
1920	52·2	1·6	57·4	2·5	58·1	2·9
1921	59·2	2·25	60·4	1·5	61·4	2·4
1922	55·9	2·06	63·2	1·7	62·3	1·1
1923	58·1	1·7	62·4	2·8	68·2	1·6
1924	59·0	2·8	62·8	1·9	67·5	2·5
1925	60·6	2·4	65·1	2·1	65·9	2·3
1926	67·1	2·49	68·1	2·8	71·2	3·04

There was a very suggestive tendency for the stocks to produce a smaller proportion of large tubers in the crop the longer they were maintained on the College Farm. Owing, however, to the variability in this respect in the crop from the separate unit plots—reflected by the somewhat high probable error—the only *significant* differences brought out by the trial are as follows:

(1) When ware sets were planted, the 1926 stocks gave a higher percentage of ware in the crop than the 1920, 1922 and 1923 stocks.

(2) When seed sets were planted, none of the differences in the amount of ware could be regarded as significant.

(3) The chat sets of the 1926 and 1923 stocks gave a higher percentage of ware than 1920. The 1923 chats were also superior in this respect than the 1922 stocks. On the other hand, owing to the high probable error of the difference of the means, there was no proof that the still greater difference between the amount of ware produced by the 1922 and 1926 chats was a significant one.

On the basis of 10 lb. samples drawn from the crops, Salaman (4) concluded that "the proportion of useful heavy ware in a crop bears a very definite and close inverse relation to the weight of the seed tuber," although elsewhere in his paper he qualifies this by the statement that the relation is not an absolute one. In the present work the probable errors were much higher than with Salaman, but the possibility of errors due to sampling were, on the other hand, avoided by analysing the whole crop in each case. Under these conditions the difference in the amount of ware produced by chat and ware sets respectively is, in most cases, not a significant one. The consistency, however, with which the small sets produced a rather higher proportion of ware than did the large sets, as is shown in Table VI, certainly supports Salaman's conclusion.

III. Chequerboard trial with Great Scot.

In 1927 a similar trial was laid down with the variety Great Scot, of which seven different year classes of stocks were then available. The same general method was adopted as with Kerr's Pink, but there were some differences in detail. After grading the tuber sets by size, they appeared so uniform in weight that the grading by weight was omitted. The unit plot consisted of single rows of sixty tubers each, and the plots were randomised in seven replications, in one of which the year classes were in "chronological" order. The different tuber classes were separated as distinct trials with the same order of planting. Tests of soil variation revealed no appreciable drift in fertility. The same method of correcting for misses in the unit plots was used as in 1926, the mean number of these misses being given for each tuber class in Tables VII and VIII.

Table VII.

Mean number of misses in each belt of plots from north to south.

Belt	1	2	3	4	5	6	7
Ware	3·1	2·1	1·7	0·9	3·3	1·4	2·9
Seed	3·6	2·1	2·1	2·0	2·1	2·1	1·9
Chats	3·1	5·3	2·7	3·4	3·4	4·7	3·6

Table VIII.

Mean number of misses in each belt of plots from east to west.

Belt	A	B	C	D	E	F	G
Ware	2·3	3·1	1·1	2·0	2·6	2·3	2·0
Seed	2·1	2·6	3·0	3·6	1·7	1·4	2·0
Chats	3·1	5·6	2·9	3·1	4·4	3·9	3·3

There is no great difference in the mortality of the sets in the tuber classes and, as with the variety Kerr's Pink, it is obvious that the misses are not affected to any extent by causes other than some inherent character of the tubers themselves.

Table IX, Column 4, gives the occurrence of misses in the various year and tuber classes and, while not so evident as with Kerr's Pink, the figures show a definite increase in the older stocks.

The planting of the trial occupied from April 25th–28th, and each series or belt was completed in one day. The crop was raised between September 20th and 27th, the delay being occasioned by intermittent but heavy rain. The whole of any one series was lifted in one day, but some series were necessarily raised when the tubers were wet and others when they were fairly dry. In order to correct, as far as possible, for this difference in the condition of the crops on lifting, the mean yield of all the year classes in a series or belt was taken to = 100, and the actual yields of the unit plots were then expressed as a percentage of this mean yield. Table IX gives the mean actual yields, the yields corrected for misses in the same way as in 1926, and finally again corrected for this

Table IX.

Year class	Tuber class	Mean yield (lb.)	Mean no. misses	Yield corrected for misses	Yield corrected for lifting	Mean P.E. of lb.	Mean P.E. of %	Mean % leaf-roll	Mean % total virus disease
1921	Ware	74·9	3·4	79·3	81·6	1·39	1·7	44·1	44·1
1922	,,	71·9	2·1	74·7	76·6	1·58	2·1	46·1	46·1
1923	,,	77·0	4·4	83·7	85·3	2·75	3·2	37·1	37·6
1924	,,	102·0	1·1	104·1	107·1	1·57	1·4	9·7	9·7
1925	,,	106·1	1·1	108·0	111·2	2·19	1·9	7·2	7·2
1926	,,	110·3	1·6	112·9	116·1	2·11	1·8	1·4	1·4
1927	,,	116·0	1·6	118·9	121·9	1·35	1·1	0·0	0·0
1921	Seed	71·7	3·0	75·5	80·7	1·75	2·2	55·2	56·4
1922	,,	69·1	2·1	71·7	76·7	1·72	2·2	54·8	55·8
1923	,,	75·7	3·1	78·3	83·3	2·88	3·4	44·8	45·7
1924	,,	96·4	3·4	102·1	109·1	1·74	1·6	13·0	14·0
1925	,,	103·3	2·0	106·8	114·0	1·77	1·5	10·8	11·6
1926	,,	106·9	1·9	110·2	118·0	3·59	3·0	2·9	3·4
1927	,,	110·0	0·4	110·9	118·0	2·42	2·0	0·0	0·0
1921	Chats	61·9	6·7	69·7	76·3	2·40	3·1	47·8	55·9
1922	,,	62·1	4·1	66·6	72·7	1·21	1·7	54·2	60·1
1923	,,	68·1	4·7	74·1	80·8	1·42	1·7	43·4	51·2
1924	,,	92·7	5·0	101·6	110·5	2·12	1·9	13·4	27·1
1925	,,	94·1	3·3	99·8	108·9	2·39	2·2	14·1	17·6
1926	,,	108·3	1·4	111·0	120·8	3·05	2·5	2·7	3·4
1927	,,	116·9	1·0	119·0	130·0	2·53	1·9	0·0	0·0

"lifting" error, so that the magnitude of these corrections can be noted. In the following pages any references to "yield" are to those as finally corrected in Column 6 of Table IX.

(*a*) *Discussion of yield results with Great Scot.*

Total yields. The distinction between the high and low yielding groups of stocks is even more marked than with the variety Kerr's Pink. It is of interest to note that only the three oldest stocks show a heavy loss, or, in other words, degeneration has been slower with Great Scot than with Kerr's Pink. This fact is brought out still more clearly in Table X, which gives the loss in each year and tuber class expressed as a percentage of the yield from the new Scotch stocks.

Table X.

Percentage reduction in total yield in 1927 *from the three tuber classes.*

Tubers planted	Year stocks were obtained from Scotland						
	1927	1926	1925	1924	1923	1922	1921
Ware	0·0	4·8	8·8	12·2	30·1	37·2	34·7
Seed	0·0	0·0	3·4	7·6	29·4	35·0	31·6
Chats	0·0	7·1	16·2	15·0	37·8	44·1	41·3

The *rate* of deterioration is clearly not dependent on the size of tuber planted for, as Table X shows, the loss from the seed sets lies between that from ware and chats.

As in the case of the Kerr's Pink trials, the significance of the differences in total yield in the various year classes has been worked out for each class of tuber planted, with closely similar results. To save space, these are given in Table XI for one tuber class only; the year classes being arranged in order of decreasing yield and the significant differences being shown in heavy type. The results of all tuber classes can be summarised as follows:

(1) All the 1921 tuber classes gave as good a yield as the 1922 and 1923 stocks, but were significantly poorer than 1924, 1925, 1926 and 1927. The parallel with the 1921 stocks of Kerr's Pink is of particular interest.

(2) The 1922 ware and seed sets produced as good a yield as the 1921 and 1923 stocks, but the chats were inferior to the 1923 chats. All tuber classes gave significantly poorer yields than 1924, 1925, 1926 and 1927 stocks.

(3) All the 1923 tuber classes were inferior to any of the younger

Table XI.

Great Scot. Significance of differences in yield and virus infection with seed-size sets.

Leaf-roll (%)	Total virus disease (%)	Yield	Year	1927	1926	1925	1924	1923	1921	1922
0·0	0·0	118·0	1927	—	*0·0* −3·4	*−4·0* −11·6	−8·9 −14·0	−34·7 −45·7	−37·3 −56·4	−41·3 −55·8
2·9	3·4	118·0	1926	*0·0* +3·4	—	*−4·0* −8·2	−8·9 −10·6	−34·7 −42·3	−37·3 −53·0	−41·3 −52·4
10·8	11·6	114·0	1925	*+4·0* +11·6	*+4·0* +8·2	—	*−4·9* *−2·4*	−30·7 −34·1	−33·3 −44·8	−37·3 −44·2
13·0	14·0	109·1	1924	+8·9 +14·0	+8·9 +10·6	*+4·9* *+2·4*	—	−25·8 −31·7	−28·4 −42·4	−32·4 −41·8
44·8	45·7	83·3	1923	+34·7 +45·7	+34·7 +42·3	+30·7 +34·1	+25·8 +31·7	—	*−2·6* −10·7	*−6·6* −10·1
55·2	56·4	80·7	1921	+37·3 +56·4	+37·3 +53·0	+33·3 +44·8	+28·4 +42·4	*+2·6* +10·7	—	*−4·0* *−0·6*
54·8	55·8	76·7	1922	+41·3 +55·8	+41·3 +52·4	+37·3 +44·2	+32·4 +41·8	*+6·6* +10·1	*+4·0* *+0·6*	—

In each group, upper figure = difference in yield in lb.
lower figure = difference in % virus diseases.

stocks. The chats gave a better yield than the 1922 chats, but otherwise the 1923 stocks were no better than 1922 or 1921.

(4) The 1924 stocks were as productive as the 1925 and the chats equalled the 1926 chats in yield. Otherwise the 1924 stocks were significantly better than older stocks and poorer than younger ones.

(5) There was no significant difference in the yields from the 1925 seed and from 1927, 1926 and 1924, but the ware and chats were definitely poorer than the 1927 stocks and as productive as the 1926 and 1924 stocks. All tuber classes were superior to 1923, 1922 and 1921.

(6) All tuber classes of the 1926 stocks gave the same yield as from the 1927 and 1925 stocks, whilst the chats were no better than the 1924 chats. All stocks were superior to 1923, 1922 and 1921, and the ware and seed gave a better yield than the 1924 stocks.

(7) The 1927 (new Scotch) stocks were definitely superior to 1924, 1923, 1922 and 1921, and the ware and chats gave a better yield than 1925. There was no significant difference in yield between the 1927 and 1926 stocks, nor between the seed of 1927 and 1925. Otherwise the new Scotch stocks gave a better yield than any of the older ones.

(*b*) *Effect of "age" of stocks on the size of tubers produced.*

As with the variety Kerr's Pink, an effort was made to determine whether the "age" of the stock planted had any appreciable effect on the proportion of large tubers in the crop, and also whether this proportion was affected by the size of tuber set used. Table XII gives the mean percentage of ware tubers produced in each of the year and tuber classes, as well as the mean probable error in each case.

Table XII.

Mean percentage of ware tubers in crop from each year and tuber class.

Year class	Ware planted	P.E.	Seed planted	P.E.	Chats planted	P.E.
1921	54·2	0·68	64·7	1·45	64·5	1·05
1922	51·5	0·80	62·4	1·63	71·4	1·70
1923	58·7	1·44	67·3	1·68	72·1	1·88
1924	60·9	1·46	71·2	0·54	77·7	1·23
1925	58·2	1·20	72·6	0·79	79·0	1·17
1926	63·8	1·27	69·8	1·59	81·1	0·84
1927	54·9	1·65	66·6	1·45	79·1	1·47

The only differences in the proportion of ware in the crop which can be attributed to the age of the stock occurred when chat sets were planted, the three youngest stocks producing a definitely larger pro-

portion of ware than the three oldest stocks. On the other hand, a comparison, year by year, of the proportion of ware from ware sets and chat sets, respectively, gives a significantly higher figure each time in favour of the chat sets, thus fully confirming Salaman's conclusion (4).

IV. OCCURRENCE OF DISEASES IN THE KERR'S PINK AND GREAT SCOT TRIALS.

Apart from virus symptoms no disease attacked either variety to any serious extent. Blackleg (*Bs. phytophthorus*) and Collar Fungus or Stem Canker (*Corticium solani*) attacks were negligible. Blight (*Phytophthora infestans*) appeared late in both seasons, and there was rather less rotting of the tubers than customarily occurs in North Wales. No appreciable difference in the intensity of attack of any of these three latter diseases was found in the various year classes, but with virus diseases the differences in degree of infection were very marked.

(a) *Definitions of virus diseases observed.*

One of the chief difficulties met with in virus disease diagnosis is the fact that well-known names have different connotations amongst workers, even in the same country, and also that what appears to be a single disease (when descriptions and photographs are scanned) masquerades under different names in literature. The analysis of symptom complexes into unit characters as proposed by Schultz and Folsom (5) is urgently needed, but it will fail in its object until adequate proof is forthcoming that simple virus diseases have been isolated. No such proof is at present available and it is impossible, with our present knowledge, to separate purely varietal reactions from the clinical picture resulting from infection by a specific virus. In these circumstances the only recourse left to the field worker is to define, as precisely as possible, the symptoms observed in the disease named.

In both the trials with which we are now concerned, each plant was examined at least twice during the months June to September, and the following diseases were noted as occurring either singly or in combination in the trial plots.

Mottling. Described plants apparently quite healthy and of normal size but which, on close inspection, showed faint mottling of one or more leaves. This appearance was practically absent from the Great Scot plots but occurred on almost every otherwise healthy plant of the variety Kerr's Pink. Its significance is discussed later.

Mosaic. Although little or no stunting was visible, the plants were obviously diseased, and the mottling was easily recognised without the aid of white paper, the leaf margins tending to be undulating. The disease occurred in almost negligible amounts in the Great Scot trial but was common in Kerr's Pink. A comparison of mosaic with the previously described "mottling" is shown in Plate XXXIV, fig. 2.

Crinkle. This much disputed name was applied to plants usually dwarfed to about two-thirds or even one-half of the normal height. The final diagnosis depended upon the occurrence of downward curling leaf margins and tips, together with blistering or corrugation of the leaf surface. Mottling was frequently faint or even invisible. Stippling of the surface with minute dark spots with brown streaking of the veins, although common, were not invariably present on plants recorded as affected with crinkle. The lower leaves usually yellowed early in the season and ultimately hung as a mass of dead, blackened foliage. This disease probably included plants which would be diagnosed as "streak" by some workers, but in no case were plants observed to show the characteristic angular necrotic areas on the leaf with the necrotic continuation into the veins as described for streak by Orton (3) and Atanasoff (1). Crinkle is illustrated in the variety Kerr's Pink in Plate XXXIV, fig. 3, and in Great Scot in Plate XXXIV, fig. 4.

Curly dwarf. The completely dwarfed appearance of the plant, due in many cases to a procumbent habit with shortened internodes and twisted foliage, made this type of degeneration easily recognised. Mottling was not invariably present. It occurred only in the oldest stocks of Kerr's Pink and not at all in Great Scot. The symptoms merged into those of crinkle, and in doubtful cases the final diagnosis depended on the degree of stunting shown. Curly dwarf is illustrated in Plate XXXV, fig. 5.

Leaf-roll. Only secondary characters of the disease were found. The lower leaves showed an upward rolling of the margin and were leathery to the touch. Mechanical injuries and the occurrence of diseases which simulate leaf-roll were of course carefully looked for before diagnosing leaf-roll. The disease is illustrated in Plate XXXIV, fig. 2.

(*b*) *Discussion on the occurrence of virus diseases in the trial plots.*

A summary of the mean percentage occurrence of these diseases in the 1926 trial with Kerr's Pink is given in Table XIII. The probable error is also given, except where the percentage disease is so low in comparison with the error that no particular significance can be attached to

the figure for the disease. As might be expected from the infectious nature of virus diseases, the older stocks were much more heavily infected than were the younger ones, but they of course contained fewer plants recorded as mottled, since, by definition, such plants were otherwise normal. The degree of significance to be attached to this mottling deserves further consideration. The tendency of workers to assume that all cases of mottling are of an infectious virus type cannot be justified. "Water-mottling" of tomatoes is not infectious, and is usually attributed to malnutrition; whilst even with known "virus-free" potatoes a form of mottling can be seen in the crumpled foliage as it emerges above ground. It may, however, be admitted that, in the present state of our knowledge, the onus of proof rests rather with the sceptic than with the believer in the infectious nature of a given case of mottling.

In the present instance a good deal of the faint mottling was observed in June and July—before any considerable transmission of mosaic was likely to have occurred, and in the following year the progeny showed only the usual moderate increase in the number of mosaic plants, instead of an almost completely diseased crop, as would presumably have been the case if most of the previous season's mottling had really represented primary mosaic infection. On the basis of such a rapid and complete transmission of mosaic to healthy plants, it would be difficult to explain why 18–20 per cent. of the oldest stocks remained free from visible infection with mosaic after seven years without change of seed.

So far as the present work is concerned, it is immaterial whether the mottling represented primary infection with mosaic or not, since it has never been suggested by any worker that potato plants suffer in yield as a result of infection exhibited as feeble or primary symptoms. Despite the faint mottling, the 1926 (new Scotch) stocks produced a uniformly vigorous crop, and the mean yield of the seven replications was taken as the standard from which was calculated the loss of yield in the other stocks[1].

[1] Even had "virus-free" material been available, the writer would not have used them in these trials, for several reasons: (1) The object of the trials would have been defeated by the use of stocks, as a standard, superior to any available to commercial growers. (2) Their use would have added an entirely fictitious scientific value to the trials since, once exposed to infection in the plots, they would immediately cease to be virus free. (3) Finality has not been reached in our knowledge of the sources of infection, or the number, of viruses which may be carried without symptoms. There might, therefore, be an undeterminable amount of such diseases conveyed to the virus free stocks, no less than to the good commercial stocks actually used.

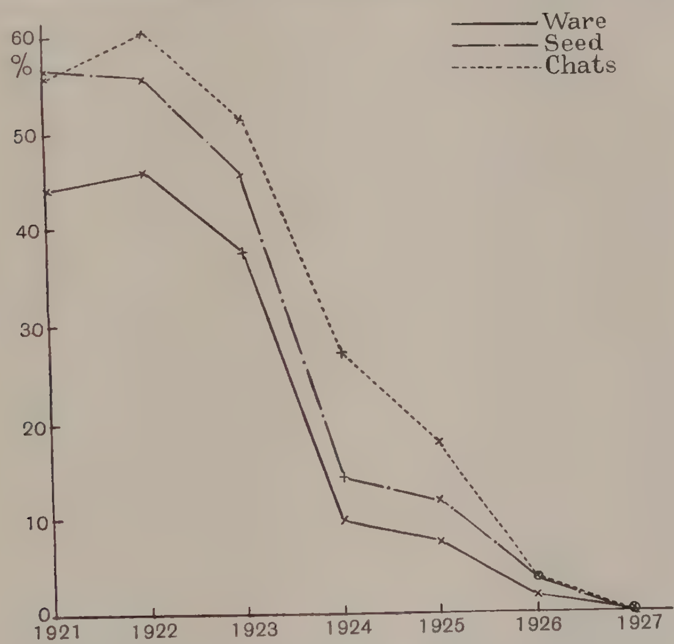
Table XIII.

Mean percentage virus diseases and P.E.'s in 1926 with Kerr's Pink.

Year class	Tuber class	Mean % mottled	P.E.	Mean % mosaic	P.E.	Mean % crinkle	P.E.	Mean % curly dwarf	Mean % leaf-roll	P.E.	Mean % total virus disease*	P.E.
1920	Ware	11·2	1·6	40·2	4·8	24·6	1·4	0·9	50·4	3·1	82·7	1·8
1921	,,	54·0	3·5	19·3	1·7	9·0	0·6	0·0	15·5	1·4	34·5	1·9
1922	,,	38·0	4·1	21·6	3·6	10·2	1·7	0·0	31·2	1·3	51·4	3·5
1923	,,	41·0	2·9	24·0	2·8	13·9	2·8	0·0	25·4	2·1	52·9	4·0
1924	,,	61·4	6·9	6·1	1·5	2·4	0·5	0·0	6·8	0·8	16·0	2·4
1925	,,	71·2	9·1	1·3	—	1·3	—	0·0	1·6	—	3·9	0·7
1926	,,	74·2	8·9	1·5	—	0·0	—	0·0	0·0	—	1·5	—
1920	Seed	13·9	1·1	39·0	6·1	17·4	2·1	1·4	54·2	3·8	82·0	2·2
1921	,,	43·7	3·3	19·5	2·8	11·7	1·3	0·0	23·4	2·9	44·9	2·3
1922	,,	38·7	4·5	28·6	3·9	13·5	2·1	0·0	28·4	3·4	53·1	4·3
1923	,,	37·9	2·8	26·9	2·8	13·5	2·1	0·0	22·5	2·1	51·1	2·4
1924	,,	61·7	7·9	6·6	—	2·1	—	0·0	6·4	—	15·0	2·3
1925	,,	65·3	9·1	5·0	—	2·1	—	0·0	2·1	—	9·2	1·4
1926	,,	76·4	9·3	0·5	—	0·0	—	0·0	0·0	—	0·5	—
1920	Chats	17·5	2·2	45·7	5·4	11·4	2·8	0·9	53·7	4·0	78·7	3·0
1921	,,	48·9	3·1	16·4	3·0	5·6	—	0·0	19·6	2·9	37·9	3·7
1922	,,	35·3	2·5	29·2	2·9	8·6	—	0·0	34·9	3·2	52·4	3·1
1923	,,	39·3	3·1	30·3	4·0	8·4	—	0·0	22·3	1·6	50·0	2·8
1924	,,	59·6	7·9	7·4	—	1·6	—	0·0	9·9	0·9	19·7	2·1
1925	,,	66·9	0·4	3·8	—	1·0	—	0·0	2·4	—	7·2	1·3
1926		81·2	7·2	0·5	—	0·0	—	0·0	0·0	—	0·8	—

* Excluding "mottling."

As regards the trials with the variety Great Scot in 1927, no mottling whatever was observed. Leaf-roll was almost the sole disease found in the ware tuber class and far outnumbered the crinkle and mosaic plants in the seed and chat tuber classes also. The percentages of leaf-roll and total virus diseases in these trials are given in the last two columns of Table IX. Graph 2, which shows the percentage of total virus diseases in each of the three tuber classes of Great Scot, makes it clear that the ware sets were less heavily infected than the chats; the difference being significant in all stocks except in 1926 and 1927. Curiously enough this is not the case when leaf-roll only is considered, for here the difference in

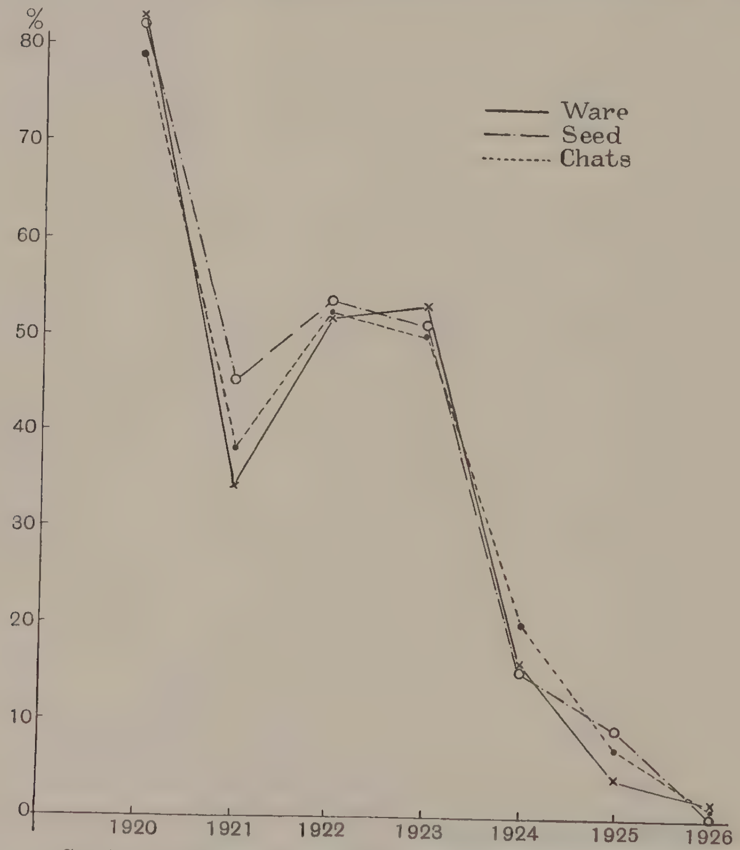


Graph 2. Percentage total virus disease (Great Scot, 1927).

infection of ware and chats becomes barely significant only in the 1925 stocks. The Great Scot chat sets were therefore no more heavily infected with leaf-roll than were the ware sets, but were definitely more heavily infected with diseases of the mosaic type. Graph 3 shows that there was no significant difference in total virus-disease infection in any of the tuber classes with Kerr's Pink, and this is also true of leaf-roll infection. From these facts we may conclude that, by planting ware tubers, no improvement in the health of the stocks was effected in Kerr's Pink, whilst with Great Scot leaf-roll infection was not affected, although

mosaic diseases were in this way considerably reduced. This bears out the conclusions reached by the writer in a previous paper (8).

The relatively low percentage of total virus diseases in all tuber classes of the 1921 stocks of both varieties is revealed in Graphs 2 and 3 respectively, whilst Graph 3 also shows the abnormally high infection of the 1920 stock of Kerr's Pink. A satisfactory explanation is not easy to



Graph 3. Percentage total virus diseases in Kerr's Pink in 1926.

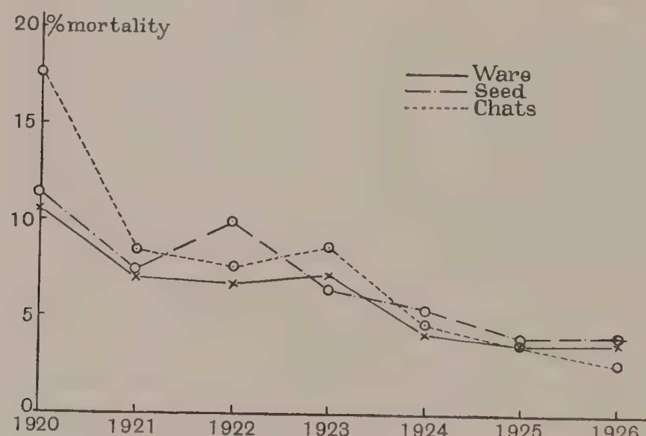
find, since the 1921 stocks had maintained their superior position relative to the 1920, 1922 and 1923 stocks in each succeeding year. We have, therefore, to postulate a difference in the degree of infection of these stocks as received from Scotland, and then to explain the persistence of this difference for seven years. Unfortunately, sufficiently detailed field counts of virus-infected plants were not made before 1924, and it is now

impossible to be definite as to the health of the oldest stocks when received. However, crop inspection in Scotland was then in its infancy, and little or no consideration was given to freedom from virus diseases, so that it is reasonable to suppose a somewhat high infection occurred in the first few years that seed was saved for the purpose of these trials. This is the more likely, since the commonest "rogues" in the early days of crop inspection were Arran Chief and Up-to-Date, the former variety, at any rate, being one almost always infected and thus serving as a source of primary infection in the seed crop in Scotland. The 1920 stock of Kerr's Pink was non-certified and was obtained as "Scotch—probably Fife grown seed" from an English merchant. All the other stocks were Class I, T.S. certified seed, obtained direct from the growers, the 1921 seed being grown on a high-lying farm in North Forfarshire and succeeding years' stocks coming from Banff. It is assumed that, by accident, an unusually healthy stock of both varieties was obtained from Forfarshire in 1921. Aphides were not recorded on the trial plots at Bangor in 1921 by Walton (7), and this is believed to be due to their having been reduced, or even exterminated, in the abnormally wet summer of 1920. There would be, therefore, little or no spread of virus diseases under field conditions at Bangor in 1921, and these stocks, when planted in 1922, would be advantageously placed as regards freedom from infection when compared with the new seed (grown in Scotland in the previous season).

Although infection regularly increased each year in the various stocks, the fact that these maintained the same relative positions, with respect to virus infection, suggests that the diseases spread more easily from plant to plant in the drill than across the drills. That this is the case has been demonstrated several times in small trials in which the progeny of plants surrounding infectors has been saved and planted the following year. Of at least equal importance is the fact that virus diseases spread rather late in the season at the Bangor College Farm. The writer has shown (8) that tubers lifted at the end of July, even when exposed to unusually great chances of infection, generally escape infection with leaf-roll. This method has been used with considerable success in restoring the vigour of a portion of the 1920 stock of Kerr's Pink when planted in the middle of a museum of virus diseases. Murphy and McKay (2), on the other hand, find that lifting must be carried out a month earlier at Glasnevin than is necessary at Bangor. Primarily infected plants at Bangor thus give a high proportion of healthy progeny, and this is undoubtedly the main reason why the stocks of these two varieties did not quickly become uniformly 100 per cent. diseased.

(*c*) *Effect of virus diseases on the mortality of seed tubers.*

It has been pointed out that the occurrence of misses in both varieties was definitely related to some inherent character of the tubers and not to their position in the plots. Graph 4 shows the gradual increase in mortality with the age of the stocks of Kerr's Pink, and a comparison of this with Graph 3 makes it probable that the reason can be found in the increase of virus infection. Salaman (4) found that chats failed more often than ware sets, and he explained this by assuming a reduction in size of tubers due to mosaic, and a consequently unconscious selection of infected tubers in his smallest tuber classes. This may be true of some

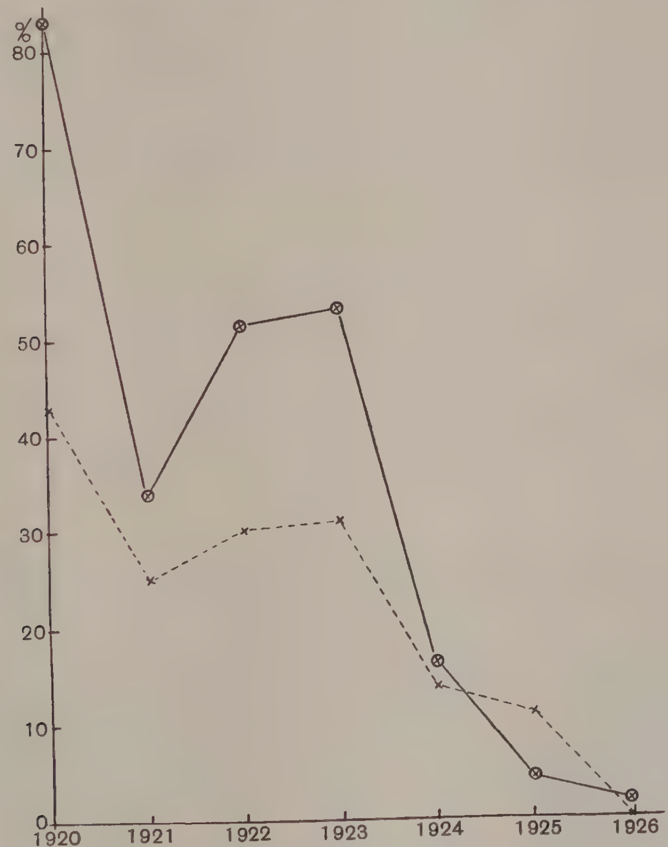


Graph 4. Percentage mortality in ware, seed and chats. Variety—Kerr's Pink.

varieties, but can scarcely be an adequate explanation in Salaman's trials, since he stated that only "some slight mosaic and no leaf-roll" occurred in his plots. In the present work there is some indication of an increase in mortality in the Great Scot chats paralleled by an increased virus infection as compared with ware, whilst in Kerr's Pink the similar virus infection of wares and chats is reflected in the approximately equal mortality in the two classes of tubers. To what extent other factors may have operated is not known, but that they cannot be ignored is evident from the great increase in the mortality of the 1920 stock of chats in the variety Kerr's Pink, which is certainly not due to any difference in virus infection between ware and chats.

V. Correlation between yield and virus disease infection.

As was stated in the introduction, the object of this work was not to add to our already ample evidence of the reduction in yield which occurs in virus-infected crops, but to try to determine the degree of



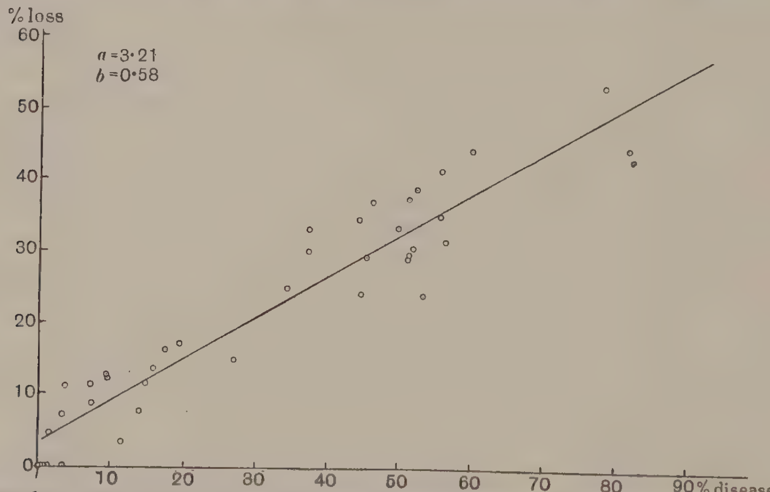
Graph 5. Kerr's Pink.

⊗——⊗ = percentage total virus disease in ware plants.
×- - - - -× = percentage loss in yield in ware plants.

relationship between them. That such a relationship exists is clear from Graph 5 in which the mean percentage loss in yield in the seven unit plots of Kerr's Pink, and the mean percentage virus infection, are plotted against each year's stock of ware sets planted. Quite similar graphs have

been obtained with the other two tuber classes of Kerr's Pink and with all tuber classes of Great Scot. This relationship is still more evident from Tables IV and XI. In these tables, not only does the arrangement of the year classes in a decreasing order of yield result in their being assembled in an increasing order of total virus and leaf-roll infection, but there is no instance of a significant difference in yield not being accompanied by a significant difference in infection[1].

In Graph 6 the loss in yield in all year and tuber classes of both varieties has been plotted against the percentage virus infection. Evidently a



Graph 6. Correlation between percentage virus disease and percentage loss in yield (Kerr's Pink and Great Scot).

definite linear correlation exists of the ordinary type $Y = a + bR$, where Y = percentage loss in yield and R the percentage virus infection. Under these circumstances it is possible to measure the interdependence of the two factors by determining the correlation coefficient with the aid of the formula $r = \frac{\Sigma(D \times D_1)}{\sqrt{(\Sigma D^2 \times \Sigma D_1^2)}}$, in which r = the correlation coefficient, D and D_1 the deviations from the mean of the loss in yield and virus infection respectively. The probable error of this coefficient was determined by the formula $\sigma = 0{\cdot}674 \frac{1 - r^2}{\sqrt{n}}$, where n = the number of observations.

[1] Where a significant difference in infection has not significantly affected the yield, it merely implies, of course, that the presence of disease to this extent could be ignored by the grower unless he proposed to save seed from the crop for planting.

The correlation coefficient, determined in this way, proved to be $= 0 \cdot 83 \pm 0 \cdot 046$ in the case of Kerr's Pink, and $= 0 \cdot 97 \pm 0 \cdot 008$ with Great Scot. It is true that these correlations have been obtained with yields "corrected" for the occurrence of misses in the case of the Kerr's Pink and, in addition, for the difference in the condition of the tubers when lifted in the case of Great Scot. These corrections, however, do not affect the conclusions, for the correlation coefficient when the mean *actual* yields are used $= 0 \cdot 79 \pm 0 \cdot 054$ with Kerr's Pink and $= 0 \cdot 98 \pm 0 \cdot 0058$ with Great Scot.

With a correlation of so high an order it is safe to conclude that factors, other than virus diseases, play only an insignificant part in the degeneration of potato stocks, or more accurately, that any such factor fluctuates with the degree of virus infection and can, therefore, be ignored by the grower.

VI. Factors influencing the rate of degeneration.

The *rate* at which a crop degenerates will be affected by any factor influencing the spread of virus diseases and increasing or diminishing their effect on the plant. The most important of these are: (*a*) Climatic factors affecting the bionomics of insect vectors; (*b*) seasonal factors; and (*c*) varietal factors which affect the reaction of the plant to aphis attack.

(*a*) *Climatic factors.*

It is generally accepted that an intimate relation exists between the occurrence of aphides and the spread of virus diseases in the crop, and therefore—as the present work shows—in the rate at which it will degenerate. A temporary effect on the aphis population may, of course, be produced by mere seasonal variations, but the more permanent climatic variations may also be responsible for such a difference in the number of aphides, over a period of years, as to enable one farmer successfully to maintain the vigour of his potato stocks for many years, when other farmers within a few miles distance are compelled to change their seed at least every two years. Several cases of this kind have been reported by the writer (9), but the best example is provided by the history of the same stocks of potatoes planted at the College Farm, Aber, and at Madryn Castle Farm Institute some 30 miles distant. The stocks of Kerr's Pink and Great Scot—the degeneration of which has been traced in the present paper—have also been maintained at Madryn Castle School since 1922 and the yields compared with that of new

Table XIV.

Cropping history of seed obtained from Scotland in the years indicated at the head of columns.
(*Taking yield of new Scotch seed each year* = 100.)

	College Farm, near Bangor									Madryn Castle Farm Institute					
Year	1920	1921	1922	1923	1924	1925	1926	1927	1928	1922	1923	1924	1925	1926	1927
	Kerr's Pink.														
1920	100	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1921	99	100	—	—	—	—	—	—	—	—	—	—	—	—	—
1922	99	99	100	—	—	—	—	—	—	100	—	—	—	—	—
1923	85	92	102	100	—	—	—	—	—	99	100	—	—	—	—
1924	47	81	83	82	100	—	—	—	—	87	96	100	—	—	—
1925	58	86	62	82	107	100	—	—	—	99	88	97	100	—	—
1926	51	95	77	73	98	98	100	—	—	105	132	100	105	100	—
1927	47	90	83	87	70	81	105	100	—	110	99	90	89	100*	—
1928	34	38	38	43	59	70	100	104	100	—	—	—	—	—	—
	Great Scot.														
1921	—	100	—	—	—	—	—	—	—	—	—	—	—	—	—
1922	—	116	100	—	—	—	—	—	—	100	—	—	—	—	—
1923	—	115	120	100	—	—	—	—	—	113	100	—	—	—	—
1924	—	84	83	88	100	—	—	—	—	124	147	100	—	—	—
1925	—	83	94	90	99	100	—	—	—	91	96	86	100	—	—
1926	—	81	97	85	111	113	100	—	—	97	92	103	87	100	—
1927	—	—	—	—	—	—	—	—	—	109	97	103	99	100*	—
1928	—	55	69	59	86	94	106	107	100	—	—	—	—	—	—

* New Scotch seed was not available in 1927 and the "once-grown" stock was taken to =100.

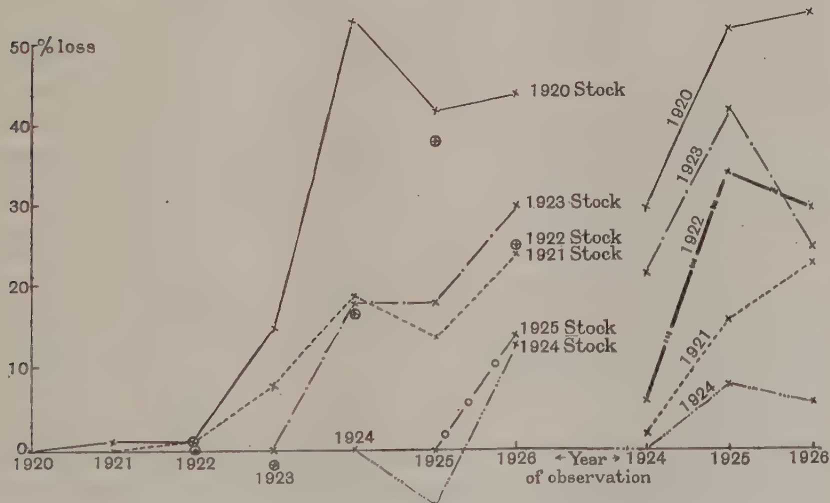
Note. No yields were recorded from the Great Scot plots at the College Farm in 1927.

Scotch seed each year. The cropping history of the stocks at these two centres is given in Table XIV[1].

The degeneration of the stocks at the College Farm is in marked contrast to the continued vigour of the same stocks at Madryn Castle. Aphides were not recorded under field conditions at the latter centre by the writer's colleague, Dr C. L. Walton, although abundant in the sheltered garden. The spread of virus diseases also was extremely slow at Madryn (10), and even in 1927 (after five years) there was only a small fraction of 1 per cent. of virus diseases present (*i.e.* two or three plants in the whole crop with either mild mosaic or aucuba symptoms). Plate XXXV, figs. 6 and 7, illustrates the vigour of the old stocks of Kerr's Pink and Great Scot respectively at Madryn in 1927.

(b) *Seasonal factors.*

These not only affect the breeding of aphides but also the vigour and possibly the resisting power of the potatoes; any one of which will in-



Graph 7. (Left) Percentage loss in each stock of Kerr's Pink (year by year) as compared with normal seed. (Right) Corresponding percentage virus disease.

fluence the rate of degeneration of a partially infected stock of potatoes. Elsewhere the writer (11) has shown that under conditions unfavourable

[1] Part of the table relating to the College Farm is taken from the Report of Experiments drawn up by Mr E. J. Roberts and published by this College in 1925. For the information in the remainder of the table the writer is indebted to Principal Isaac Jones and Mr Edwin Jones of the Madryn Castle Farm Institute, South Carnarvonshire.

for the growth of potatoes, leaf-roll plants suffer more than do healthy ones, so that the loss is more evident. It is true that, in any one year, a partially diseased stock will give a smaller yield than a healthy one grown under identical conditions, but it by no means follows that a progressive increase in virus infection over a number of years will be paralleled by a progressive decrease in yield as compared with healthy plants. Graph 7 shows that in 1925—a particularly good year for potatoes—there was a distinct improvement in the yield of the old stocks of Kerr's Pink at the College Farm, although virus infection had very much increased. This no doubt affords a partial explanation of the discrepancy in the results of variety trials in which the same stocks are planted in widely separated localities, for in some centres the seasonal factors may affect the yield of diseased plants favourably; in other, adversely.

(*c*) *Varietal factors.*

The influence of varietal factors on the rate of degeneration is well illustrated by a museum of some forty varieties infected with one or more of almost all known virus diseases, which the writer has maintained since 1924[1]. Each year, short rows of normal plants of many of these varieties have been planted in the museum, so that they have been exposed continuously to infection from all the diseases, and the stages in their degeneration could thus be followed. Most of the varieties quickly became mottled but, whilst some degenerated rapidly into the curly dwarf form, others remained predominantly leaf-roll or crinkle, and one or two are still vigorous and apparently normal.

This difference in reaction certainly affects the rate of degeneration. It is usually explained as a difference in susceptibility, but if so, we must distinguish between (*a*) liability of haulm to infection, (*b*) liability of tubers to contract infection from the diseased haulms, and (*c*) the effect on the yield. An attempt was made in 1924–5 to separate these different forms of susceptibility with very suggestive results, although of course it is probable that some of the conclusions reached would be modified if the trials could be repeated over several seasons under different environmental conditions. Twenty-five tubers each of twenty-three varieties were halved and corresponding halves were planted in the one case between leaf-roll plants and in the other between mosaic infectors. Each half tuber thus acted as a control for the other half, and the appearance of the same symptoms in the two halves of the same tuber caused the

[1] For most of the original infected materia in the virus museum the writer is indebted to the Scottish Plant Registration Station Corstorphine.

plants to be discarded from both plots. Several varieties were deleted from the trial in the first year (1924) owing to the occurrence of more than one or two diseased plants. In 1925 the whole progeny from each plant of the remaining varieties was separately planted and notes were taken of the appearance of symptoms during June to August. Finally, the produce from each plant was counted and graded, and the weight compared with that from a similar number of normal plants of the same variety growing in the same plots.

(*d*) *Mosaic.*

The infectors in 1924 were selected from the progeny of a stock of mosaic infected Irish Chieftain, amongst which were a very few curly dwarfed plants. In 1924 quite 80 per cent. of the tubers gave rise to curly dwarf plants, and mosaic was transmitted very irregularly to the varieties under test. It is believed that this irregular transmission was due to aphides migrating from one healthy row to another and so escaping infection from the intervening dwarfed rows.

(*e*) *Leaf-roll.*

Table XV gives the percentage infection of the plants in 1924 (as shown by the occurrence of one or more diseased plants from their progeny in 1925), the percentage of disease in the progeny of the infected 1924 plants—thus giving a measure of the liability of the tubers to contract disease from the infected haulm in 1924, and, finally, the percentage loss in yield (weight and numbers of tubers) of the diseased stock in 1925 as compared with healthy plants in the same plots.

It is of course possible that some of the differences in percentage haulm infection may be due to accidental causes such as a haphazard distribution of aphides, but it is unlikely that differences of this magnitude can entirely be explained in this way. A far more probable explanation is a difference in palatability or in shelter for the aphides. The difference exhibited by varieties in the percentage of tubers contracting disease from the infected haulms points to a difference in the time at which the varieties became infested with aphides. This may not be merely a matter of calendar date, but of time in relation to tuber formation. On the other hand, the possibility of a difference in the rate of movement of the virus through the haulm of different varieties cannot be excluded, and it is evident that much further work on these lines is desirable.

From the growers' point of view, susceptibility is mainly a question of the reduction in cropping power, as measured by the total weight and

Table XV.

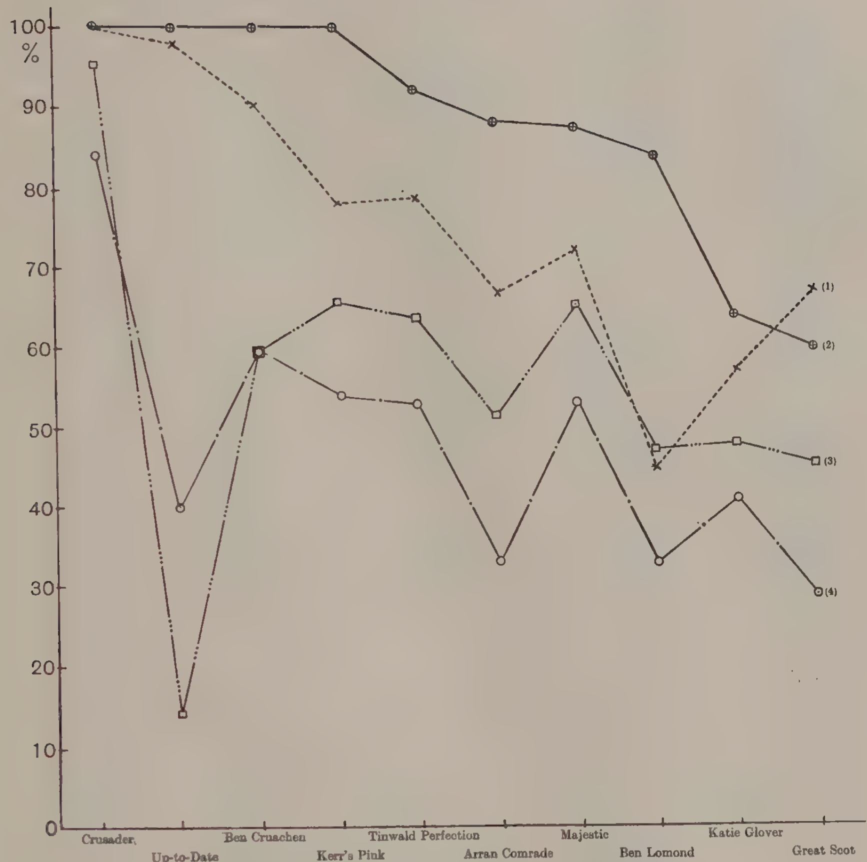
Susceptibility of varieties to leaf-roll infection (1924).

Variety	% plants infected (1924)	% leaf-roll tubers on infected plants	% loss in yield Weight	% loss in yield Numbers	Notes
Crusader	100	99·5	95·0	84·0	Very stunted
Up-to-Date ...	100	98·0	14·0	40·0	Stunted
Ben Cruachen ...	100	90·6	60·0	60·0	Very stunted
Kerr's Pink ...	100	78·1	65·0	54·0	Stunted
Tinwald Perfection	92	78·5	63·0	53·0	Stunted
Arran Comrade ...	88	65·1	51·0	33·0	Stunted
Majestic	87	70·7	65·0	53·0	Stunted
Ben Lomond ...	84	44·4	47·0	33·0	Slightly stunted
Katie Glover ...	64	54·5	47·0	41·0	Slightly stunted
Great Scot ...	60	65·5	45·0	29·0	Slightly stunted
"Sarn"*	100	94·5	—	—	Stunted
Skerry Blue ...	72	27·1	—	—	Slightly stunted
Shamrock... ...	4	84·6	—	—	Slightly stunted
Flourball	0	0·0	—	—	Not stunted

* The variety "Sarn" was said to have been brought from Russia about 1898 and, since then, had been grown at Sarn, Carnarvonshire. It is an Abundance type and, when grown at the College Farm in 1924, appeared normal and very vigorous.

size of tubers produced by the infected plant. From Table XV we find that an all-diseased stock of Crusader yielded 95 per cent. less than a normal stock, whereas a similar stock of Up-to-Date suffered only to the extent of 14 per cent. loss by weight. Again, although most of the varieties showed a decrease in the size of tubers as well as in the number produced by infected plants, the tubers from the leaf-roll Up-to-Date plants were actually larger than those produced by normal plants. It is possible that these facts help to account for the long-continued popularity of this variety. The relation of the liability of haulm and tubers to infection, and the effect of leaf-roll on the yield, are shown in Graph 8.

An attempt has been made in Text-fig. 1 to analyse the crop from the different varieties. The percentage infection with leaf-roll is shown in each tuber class from all the plants, whether they contracted or escaped infection in 1924. This gives a measure of the net effect of haulm and tuber susceptibility and confirms a conclusion reached by the writer in a former paper (8) that no improvement in the health of a partially diseased crop can be expected by selecting, as sets, any particular size of tuber. Table XVI gives similar information and, in addition, shows the proportion, by number, that each class of tuber bears to the whole crop. It illustrates the difficulties in the way of giving useful advice to growers



Graph 8. Varietal susceptibility to leaf-roll in 1924.

(1) Percentage progeny from infected plants. ×- - - - - - - - -×

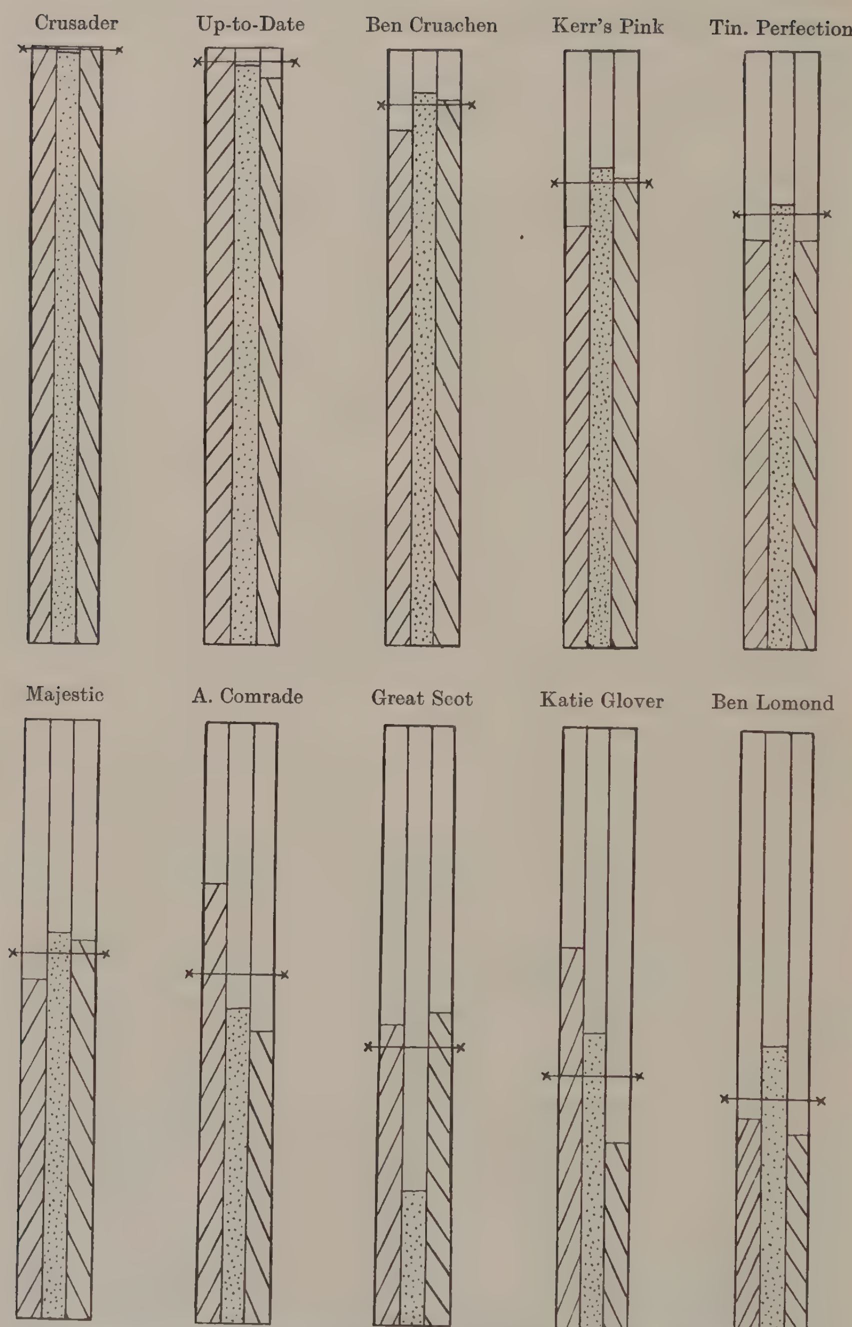
(2) Percentage plants infected in 1924. ⊕————⊕

(3) Percentage loss in weight of tubers. □—— • • • ——□

(4) Percentage loss in number of tubers. ○———•———○

Table XVI.

Variety	% of total crop			% leaf-roll in each tuber class		
	Ware	Seed	Chats	Ware	Seed	Chats
Crusader	14·2	44·2	43·5	100	98·7	100
Up-to-Date ...	34·1	35·3	30·6	100	97·7	95·5
Ben Cruachen ...	19·7	39·9	40·4	86·1	92·5	91·9
Kerr's Pink ...	18·3	42·6	39·0	70·5	80·3	79·2
Tinwald Perfection	9·6	53·2	37·1	68·9	74·6	68·8
Arran Comrade ...	23·6	40·7	35·7	73·0	52·3	48·2
Majestic	44·1	28·4	27·5	56·5	64·4	63·2
Ben Lomond ...	17·9	39·8	42·3	35·5	46·7	32·4
Katie Glover ...	12·7	41·1	46·2	63·3	48·4	31·2
Great Scot ...	39·9	28·6	26·4	50·0	21·9	52·0



Text-fig. 1. Percentage leaf-roll in ware ▨, seed ▒ and chats ▧

×——× = mean percentage leaf-roll.

as to the relative value of popular varieties. Up-to-Date and Great Scot, for instance, deserve equal commendation as regards the production of large tubers in the crop, but had seed been saved for planting, practically all the succeeding crop of Up-to-Date would have been leaf-roll whilst only some 22 per cent. of the Great Scot seed would have perpetuated the disease. On the other hand, the loss from the infected seed of Great Scot would have been much heavier than that from the infected Up-to-Date.

Murphy and McKay (2) have expressed the opinion that the probable duration of life of a variety is not conditioned by its liability to contract —and show symptoms of—virus diseases, but rather by its reaction to these diseases after infection. With this opinion the writer is in full agreement, and the present work provides strong evidence in its support. It is quite otherwise with the view expressed in the same paper that the susceptibility of varieties cannot adequately be tested until virus-free stocks and pure diseases are available. Such a view does not differentiate between the fundamental aspects of the problems and the legitimate claim of growers for guidance as to the probable result of planting different varieties exposed to similar chances of infection under natural conditions. Tests of the reaction of plants to virus infection induced by grafting, or the use of insect vectors under cages, will never replace the multiplicity of field conditions which determine, not only whether the haulm and tubers contract infection, but also the effect of infection on the yield. True, field trials must necessarily ignore the presence of "carrier" plants, but in this respect virus-free stocks—planted in the field—have no advantage over the use of normal, vigorous stocks. All recent work shows that symptomless "carrying" is an exceedingly common phenomenon in potato varieties, even when, as in the writer's view, one excludes all cases of mere "masking" of symptoms due to environmental conditions. There is, indeed, much to be said in favour of the deliberate encouragement of the breeding of perfect "carrier" varieties and the elimination of "intolerant" kinds from the market.

VII. ACKNOWLEDGMENTS.

The writer wishes to express his indebtedness to Prof. R. G. White for the facilities afforded, and the constant interest he has shown in this work. Also to Messrs O. R. Morris, D. Green, M. R. Roberts and G. L. Turner for the assistance they have given, at various times, in carrying out the field trials.

VIII. Summary.

1. Trials carried out at the University College Farm, near Bangor, N. Wales, have demonstrated the intimate relation of virus disease infection to the fall in yield experienced when potato "seed" is saved more than one year. These trials were laid down with seed tubers of three sizes corresponding to commercial "ware," "seed" and "chats." Two varieties were used, viz. Kerr's Pink and Great Scot, which had been grown for varying periods, up to seven years, without change of seed.

2. Whatever size of seed tuber was used, the yield became progressively less from the youngest to the oldest stocks, except that grown for six years, which compared well with slightly younger stocks. Again, with the exception of the six-year-old stocks, the percentage of plants free from virus symptoms fell consistently from the youngest to the oldest stocks. The continued relative superiority of these six-year-old stocks of both varieties is considered to be due to (*a*) exceptional freedom from infection when received from Scotland, (*b*) the spread of disease more easily along the drills than across them, and (*c*) the relatively late infestation by aphides which occurs at the College Farm and results in a large number of tubers on primarily infected plants escaping infection—the effect of (*b*) and (*c*) being to maintain any original difference in infection between stocks. The possibility of a difference in the reaction of different strains of a variety to virus infection cannot be ignored but is regarded as unlikely in the present instance.

3. The relationship between virus infection and loss in yield has been shown to be a linear correlation, and that it is of an extremely high order is evident from the fact that the correlation coefficient in the case of the Kerr's Pink is shown to be $0{\cdot}83 \pm 0{\cdot}046$, and with Great Scot $0{\cdot}97 \pm 0{\cdot}008$.

4. "Misses" occurred to the extent of about 5 per cent. in the youngest stocks but increased progressively in the older ones, irrespective of the position they occupied in the chequerboard plots. There is, therefore, another source of loss, which has not hitherto been generally recognised, to be attributed to prolonged home-saving of seed, and probably—though not certainly—to virus infection.

5. The effect of virus diseases on the size of tubers produced has been studied. There is no clear evidence in either variety of any marked reduction in size attributable to virus infection, but definite evidence that the proportion of large tubers in the crop is affected by the size of set planted.

6. No evidence was found that the rate of degeneration was affected by the size of tuber set planted, except possibly in the oldest stock of Kerr's Pink. In the variety Kerr's Pink there was no significant difference in virus infection in the three tuber classes, however long they had been grown without change. This was almost equally true of Great Scot so far as leaf-roll was concerned, but the chats produced a higher percentage of mosaic and crinkle plants than did the ware.

7. The effect of climatic, seasonal, and varietal factors on the rate of degeneration has been studied:

(*a*) A detailed comparison has been made of the vigour of the same stocks of two varieties maintained at the College Farm, near Bangor, and at Madryn Castle Farm Institute some thirty miles distant. Owing probably to climatic and topographical factors, aphides occurred only sparsely at the latter centre and practically no reduction in vigour in the six-year-old stocks occurred.

(*b*) Seasonal factors, by influencing the rate of breeding of insect vectors and the vigour of the potatoes, may considerably accelerate or retard the degeneration of a partially infected stock. It is shown that, owing to the difference in the response of healthy and diseased plants to unsuitable conditions, it may happen that a heavily diseased stock in an exceptionally good growing year will show less loss relative to the crop from a healthy stock than it did in a previous year in which less virus disease was present.

(*c*) The influence of variety on the rate of degeneration is shown to be the resultant of three separate forms of susceptibility, *i.e.* liability of haulm to infection, liability of tubers to contract infection from the diseased haulm, and the effect of infection on the yield. The variety Crusader showed all three forms of susceptibility in an extreme manner; Up-to-Date was very susceptible to infection both of haulm and tubers, but the virus infection had relatively little effect on the yield; on the other hand, the variety Great Scot exhibited considerably less susceptibility of haulm and tubers to infection, as compared with Up-to-Date, but appreciably more effect was produced on the yield.

The value to be attached to field trials of susceptibility to virus diseases is discussed.

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EXPLANATION OF PLATES XXXIV AND XXXV

PLATE XXXIV.

Fig. 1. Normal plant in the seven-year-old stock of Kerr's Pink, in the 1926 chequerboard trials at the College Farm, near Bangor.

Fig. 2. Leaves from plants of Kerr's Pink, showing (*a*) mottling, (*b*) mosaic, (*c*) leaf-roll.

Fig. 3. Crinkle in Kerr's Pink photographed to same magnification as Fig. 1, and from the same stock.

Fig. 4. Normal (right) and crinkle plants of Great Scot.

PLATE XXXV.

Fig. 5. Curly dwarf plant in the variety Kerr's Pink, photographed to the same magnification as Plate XXXIV, figs. 1 and 3, and from the same stock.

Fig. 6. "Age" of "seed" trial with Kerr's Pink in 1927 at Madryn Castle Farm Institute. Once grown seed on right and six times grown seed on extreme left; single rows of each year's seed.

Fig. 7. As for Fig. 6, but the variety is Great Scot and the oldest stock is on the right.

(*Received October 26th*, 1929.)

Fig. 2.

Fig. 4.

Fig. 1.

Fig. 3.

WHITEHEAD.—A Study of the Degeneration of certain Potato Stocks (pp. 452–486).

NOTES ON THE CULTURING OF INSECTS FOR VIRUS WORK

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(With Plate XXXVI and 3 Text-figures.)

I. The use of cellophane for breeding cages.

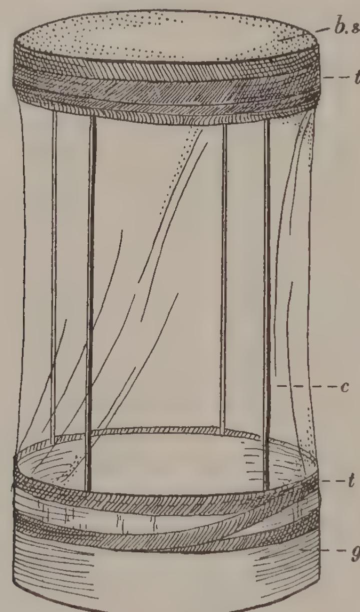
Not the least important of the difficulties faced by the breeders of insects for virus experiments is the necessity for maintaining stocks unmixed and free from infection. As a general rule it is impossible to keep each set of insects in a separate glasshouse chamber, and, in any case, it is obvious that no chamber is "insect proof" if people pass in and out. The insects have therefore to be kept in cages which must possess certain characteristics, *i.e.* they must be portable, airy, light (transparent) and minutely insect proof, as many of the suspect insect vectors are very small and ubiquitous, *e.g.* thrips, white fly, red spider.

The materials most generally used are muslin, glass, or a combination of both, but these have many disadvantages. Muslin is not easy to handle satisfactorily and harbours eggs and small insects in its folds. If it is fine enough to keep out very minute pests it cuts off a great deal of light, a failing particularly noticeable in the winter months, and even coarse muslin has to be removed for observation of the culture. Glass is fragile; for large cages it is unwieldy (and also expensive), and for small ones causes a high percentage of humidity which is bad for many kinds of cultures, especially over long periods.

Text-fig. 1 shows a type of cage which overcomes some of these difficulties. Its basis is the usual type of light metal frame, consisting of four upright wires supporting two galvanised iron bands, $1\frac{1}{2}$ inches and 3 inches deep respectively. A useful size is 14 inches height by 7 inches diameter, but the dimensions can be modified in accordance with the purpose of the cage. The walls are of cellophane[1] and it is roofed by fine bolting silk[2]. Cellophane is a material which is becoming invaluable to the entomologist. It was first brought to my notice by Dr A. D. Imms,

[1] The Cellophane Co., 7, 8 and 9 Bird Street, London.
[2] Dufour Bolting Silk, Henry Simon, Ltd., 20 Mount Street.

who had observed its use in America. Dr Imms has also been helpful with other suggestions, including information on the subject of bolting silk. Cellophane is a cellulose composition, light, durable, perfectly transparent and non-porous, though it allows the diffusion of water-vapour and other gases (3). Smith (3) gives a description of its behaviour with regard to water-vapour, and mentions its permeability to ultra-violet light which is said to be the same as that of quartz glass.



Text-fig. 1. Cellophane-covered cage for culturing aphides on small cabbage plants. *b.s.* Bolting silk cover. *c.* Cellophane. *g.* Galvanised iron base. *t.* Insulating tape binding.

Incidentally, as a material it is decidedly cheaper than any other, except perhaps the very coarsest of cheese-cloths and muslins.

These cages are easy to handle and occupy a minimum of space in the glasshouse. Perhaps the best method of construction is as follows—starting from the finished wire frame which can be made by the local ironmonger or tin-smith, the metal bands are smeared with a waterproof cement, which can be made by dissolving cellulose acetate in ethyl acetate: similar preparations are sold in tubes like seccotine[1]. A sheet of

[1] "Pear Drop" waterproof and household cement, the Turnbridge M.F.G. and Supply Co., The Nurseries, Tangley Road, Tooting, S.W.

cellophane of suitable size is quickly and not too tightly wrapped round, leaving plenty of margin at the top and the bottom. If it is too tight it is liable to split, as cellophane shrinks when damp. The free edges are then joined by a liberal application of cement, and a circle of bolting silk is gummed round the edges and stretched over the top by an elastic band. It is then finished by a tight strapping of ordinary 1-inch insulating tape which covers the free edges of the material, and is arranged so as to protect the cellophane where it is drawn over the sharp metal edge. As the insulating tape is only intended to stick to itself, the strapping must be made to overlap. If, as shown in Text-fig. 1, only two bands are used at the base of the cage, the inner free end should be well covered and the strapping should be finished on the upper edge of the metal so that the outer free end is above the level of the water in which the cage stands. It is advisable to cement down the outer edge, as the rubber solution tends to rot.

The cages stand in damp sand (for aphid) or water (for thrips as they might pupate in damp sand) in any flat receptacle of convenient size. Large earthenware plant-pot saucers are useful for holding the smaller cages. The whole can easily be removed from the chamber to be opened, but it is not necessary to open the cages for general observation purposes. The cellophane lasts about three months in a moist atmosphere, but it is safer to replace it about every ten weeks.

Plate I, fig. 1, shows the cages being used in a greenhouse in which feeding experiments and small cultures under lamp glasses are also being carried out.

II. ARTIFICIAL FEEDING OF *MYZUS PERSICAE*.

It is of great importance in many problems of virus entomology to rear the insect vector apart from the host plant. This type of work has already been carried out by Severin and Swezy (2) and Swezy (4) for *Euttetix tenella*, using the methods of Carter (1). The apparatus consists of a bag of "fish skin" (swim bladder) suspended in a cage and containing the medium which the *Euttetix* pierces the membrane to obtain. So far, however, it has not been used to culture aphides, which are also important as vectors of virus diseases. The Carter apparatus fails in that the aphides always crawl up to the roof of the cage and do not seem to recognise the damp globular surface as a substitute for a leaf. Obviously it is necessary to devise some means whereby the aphides may feed more or less in their natural position, which is upside down on the lower surface of a leaf.

Text-fig. 2 shows the first type of apparatus used. It is described in detail as, though limited in capacity, it is an easily prepared device and quite effective for certain types of work. It consists of a small crystallising dish (diameter $1\frac{1}{2}$ inches) containing a little feeding fluid closed with a circle of washed fish skin held in place by an elastic band. A filter funnel (diameter 2 inches) is prepared for its reception by the construction of a small shelf of paraffin wax and the introduction of a few aphides,

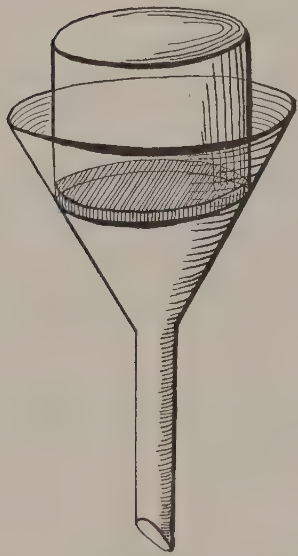


Fig. 2.

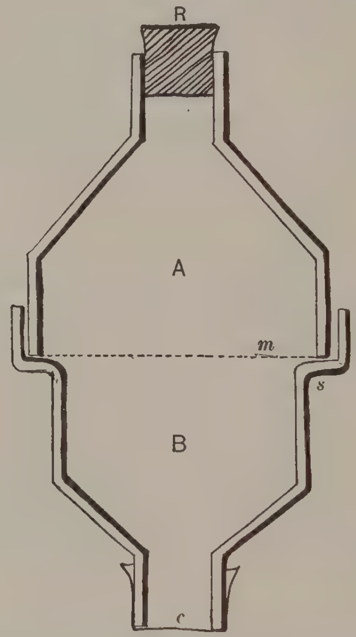


Fig. 3.

Text-fig. 2. Artificial feeding of *Myzus persicae*. Feeding apparatus, No. 1. Crystallising dish inverted over filter funnel.

Text-fig. 3. Artificial feeding of *Myzus persicae*. Feeding apparatus, No. 2. The crystallising dish and funnel replaced by specially constructed capsules.

the top of the stem being plugged with cotton-wool. The dish is inverted into the funnel and pressed down and the whole clamped in position. In this way aphides have been fed for six days on weak methylene blue and sugar solution and have produced young. This method, however, is not suitable for work on a large scale. Only a few aphides can be introduced as they cannot be kept in an open filter for more than a few seconds; also the apparatus cannot be kept in a glasshouse as it is very prone to "sweat" at high temperature. Only mixtures which are not likely to

decompose can be used because there is no way of replacing them, and extracted plant juices quickly break down and possibly become toxic to the insects. It is also advisable to use a very thin film, adding distilled water as it evaporates, pressure on the membrane causing it to leak. This cannot be done with a sealed capsule. On account of these difficulties the apparatus shown in Text-fig. 3 has been devised.

This apparatus consists of a pair of cone-shaped glass capsules *A* and *B*, their apices forming wide necks. *A* stands on a ground-in shelf (*s*) in *B*. It is covered with fish skin (*m*) as in the first apparatus (if fish skin is not obtainable gut skin can be used)[1] and contains the feeding fluid. By removing its rubber stopper (*R*) liquid can be changed or replenished without removing the capsule. Capsule *B* is perforated by four holes of 1 cm. diameter, some of which can be seen in the photographs (Plate XXXVI, fig. 2). They are covered with small circles of organdie muslin. In introducing the aphides the apparatus is inverted so that *B* is resting on *A*. The insects are placed on the membrane with a fine camel-hair brush through the neck of *B* which is then closed by a cap of muslin or cellophane (*c*). From 50 to 100 aphides can be introduced in this way without escapes. The apparatus is reversed and clamped into position as shown in the photograph (Plate XXXVI, fig. 2).

In replenishing the liquid the cage is inverted and the stopper removed. When the aphides have crawled into the upper part of *B*, the membrane can be rinsed with distilled water from a wash bottle. It is sometimes advisable to paint the edges of this membrane, when dry, with hot paraffin wax, as oozing takes place most frequently round the edge.

The mortality of *Myzus persicae* cultured in this way varies considerably with the strength and nature of the fluids and with the general conditions. Six days is the longest period for which they have remained alive (seven days in some cases for methylene blue), but it is hoped that the time will be much longer when the correct medium has been found. Using extracted potato juice the attempt to subculture on to seedlings is generally unsuccessful after the third day, though a few survive after the fourth. The subcultured aphides require a small glass cage with high humidity. That the insects actually feed and do not merely exist in the moist atmosphere is shown by the fact that stain can be detected in the gut after a few hours and many eventually become quite deeply coloured.

Table I shows the results of one set of cultures, but the numbers are

[1] Gut skin is a good deal thinner than fish skin and only the toughest pieces should be used. It can be obtained at Boots Cash Chemists, by special order.

very variable, *e.g.* in methylene blue cultures the numbers remaining alive vary from 10 out of 12, to 7 out of 50, in six days. It represents fairly well the value of the different fluids as culture media.

Table I.

Number of aphides alive on consecutive days.

Medium	Days 1	2	3	4	5	6	7
Potato juice 25 % in distilled water	20	15	11*	8*	—	—	—
Methylene blue 0·05 % in sugar solution 0·05 %	20	17	15	12	8	5	3
Dahlia violet 0·01 % ...	20	18	16	16	13	10	—
Eosin 0·01 %	20	18	15	14	11	11	—
Light green 0·02 % ...	20	14	13	11	10	9	—
Eosin azure 0·01 % ...	20	13	7	3	0	—	—

* Leaf-juice cultures were not carried beyond the third or fourth day as it was found impossible to subculture the two or three weakly specimens that survive till the fifth or sixth day. Eosin and methylene blue insects can be subcultured quite satisfactorily on the fifth or sixth day.

Summary.

1. A method for keeping pure and uninfected cultures of aphides for virus work is described; it involves the use of cellophane and bolting silk on a metal framework.

2. Specially constructed glass capsules are described in which aphides can be fed on artificial media, plant extracts or dyes.

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EXPLANATION OF PLATE XXXVI

Fig. 1. Cellophane-covered cages on glasshouse staging.
Fig. 2. Feeding apparatus, No. 2, in use.

(*Received March 3rd*, 1930.)

Fig. 1.

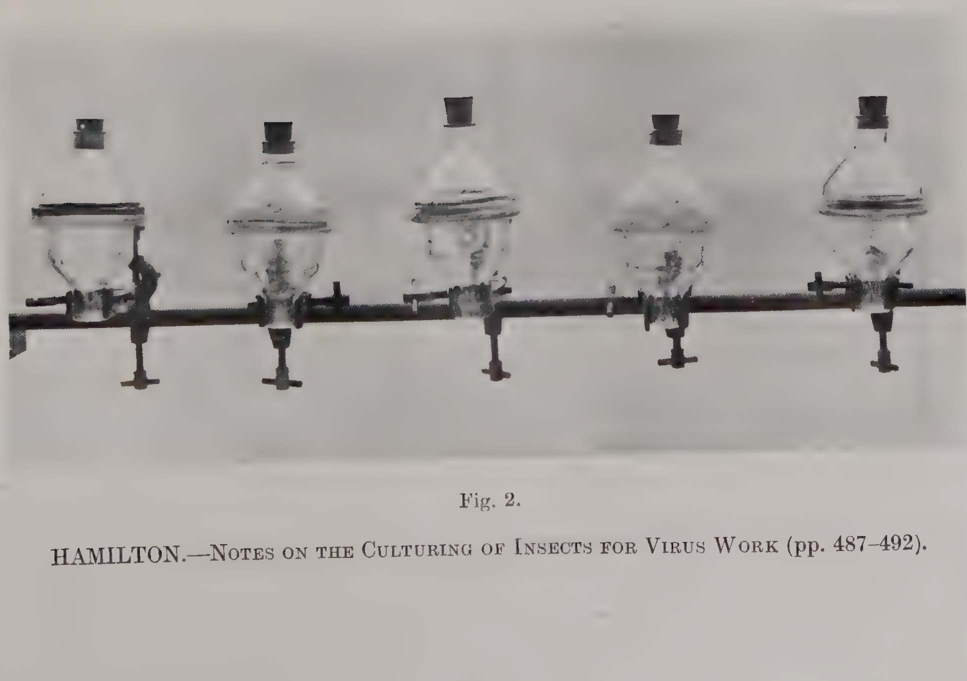

Fig. 2.

HAMILTON.—Notes on the Culturing of Insects for Virus Work (pp. 487–492).

THE INFLUENCE OF ENVIRONMENTAL CONDITIONS ON THE DEVELOPMENT OF THE ANGULAR LEAF-SPOT DISEASE OF COTTON. II. THE INFLUENCE OF SOIL TEMPERATURE ON PRIMARY AND SECONDARY INFECTION OF SEEDLINGS

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In an earlier paper (4) were given the results of a preliminary investigation of some of the environmental factors which influence the development of the disease of cotton variously known as "Blackarm," "Angular Leaf-Spot," "Bacterial Boll-Rot," etc., caused by *Bacterium malvacearum* E.F.S. (*Pseudomonas malvacearum*). It was shown that the conditions of air temperature and humidity each influenced the development of the disease resulting from artificial inoculation of young plants, and that the factors were interrelated in the sense that a change in one allowed of a change in the other without effect on the disease. No attempt was made to differentiate between soil and air temperature, although it was recognised that such a distinction must be made in a more complete investigation of the problem. This was especially true in view of the results of Massey (2) who, working in the Sudan, claimed that the development of the disease in the seedling stage was confined to a definite range of soil temperature, namely from 11° C. to 28–30° C., definite immunity being obtained at 32° C. In a later paper (3) the same author produced further evidence of this influence of soil temperature, and recapitulated the evidence for internal infection of seed first suggested by Archibald (1), but elaborating the theory to include systemic infection of the entire plant under certain conditions without external manifestation of disease.

Preliminary attempts by the writer to confirm these results proved negative, but the question was of such obvious importance that a request from the Sudan Authorities to the Empire Marketing Board for a grant to assist the work was acceded to, and under this grant a series of special chambers was constructed in which soil temperature, air temperature, air humidity and illumination could be independently controlled. A detailed account of the apparatus has been published (5), and it is unnecessary to give full details here. Each unit consists essentially of a

heat-insulated and thermostatically controlled water-tank, in which the soil tins for the plants are suspended, and fitting over this tank a double-walled glass air-chamber, the temperature and humidity within which are automatically controlled. Illumination is provided by two 500-watt lamps in suitable reflectors suspended over each case, the heat from the lamps being absorbed by a continuously flowing water film. Cotton seedlings have been found to grow satisfactorily in these chambers, although owing to the entirely overhead character of the lighting and its low intensity compared with tropical sunlight they become somewhat "drawn."

The influence of several factors has been investigated to some extent, but only those experiments dealing with the effect of soil temperature on seedling infection will be described here. Later papers will deal with the other factors, especially with regard to their influence on secondary infection by spray inoculation.

It should be pointed out that so far no attempt has been made to deal with the extremely difficult problem of automatic control of soil moisture. Throughout the experiments the plants were supplied with water of the right temperature from time to time as thought necessary. Examination of the soil at the end of the experiments showed that in no case had watering been so excessive as to cause any degree of waterlogging. While it is recognised that soil moisture may have some influence on the disease either directly or indirectly through its effect on the plant, the consistency of the results with different types of soil and the fact that the moisture was kept well within the extremes of dryness and wetness makes it improbable that the results will be seriously affected by variations in this factor.

The seed used throughout the experiments was "Sakellarides" variety from the Gezira Plain, supplied by the courtesy of Mr R. E. Massey, Botanist to the Sudan Government. Different lots of seed were used in each experiment, but all the seed was derived from heavily infected plants, and hence presumably carried the organism.

Description of experiments.

Influence of soil temperature on primary infection.

Exp. 1. Four different treatments were used in the experiment, and as there were eight tins in each chamber, each treatment was duplicated at every temperature. Ten seeds were sown per tin, giving a possible twenty seedlings per treatment at each temperature. The treatments were as follows:

(*a*) Seed delinted in concentrated sulphuric acid for 15 minutes, washed, immersed in 1 : 500 mercuric chloride for 15 minutes, and again washed.

(*b*) Seed soaked in a very heavy suspension in distilled water, from a pure culture of a virulent strain of *B. malvacearum*.

(*c*) A small portion of the testa of each seed carefully chipped off from the side without injury to the embryo and the seed then soaked in a suspension of the organism.

(*d*) Seed soaked in sterile water, but otherwise untreated.

In each case the vessel containing the seed and disinfectant, suspension, or water respectively was evacuated for a short time to ensure complete wetting of the seed. After treatment the seed was sown directly in the tins at a depth of about 4 cm., at the following range of temperatures: 15° C., 19° C., 23° C., 27° C., 31° C., 35° C. The soil used was a rich glasshouse compost containing one-fourth of its bulk of sand. The temperature in all the air-chambers was maintained at 25° C. and the humidity at an average of 75 per cent. As soon as the seedlings appeared above the soil the artificial lighting was provided, the time-switch being set to give 16 hours' illumination daily.

Germination was good at all temperatures except 15° C., where the seed germinated irregularly and sparsely. The times required for germination at the different temperatures are shown in Table I, where the time given refers to the first appearance of seedlings above the soil.

Table I.

Time (in days) for germination and germination percentage.

	15° C.	19° C.	23° C.	27° C.	31° C.	35° C.
Time for germination	5–6	5	3	2½	2	3
Total no. germinated	25	54	73	66	67	74
Germination %	31·3	67·5	91·3	82·5	83·8	92·5

The "number germinated" and "germination percentage" given in Table I include all seed irrespective of treatment, that is, a total of eighty seeds at each temperature.

Infection was apparent a few days after germination, and an examination of the seedlings was made 14 days after sowing except in the cases of chambers numbers I and VI (19° C. and 15° C.), when the examination was made 19 days from the date of sowing. Infection of the cotyledon takes the form of water-soaked spots which are usually most numerous around the edges of the cotyledon. Any seedling showing one

or more definite lesions of this type was counted as infected, and in Table II the numbers of such infected seedlings are given for the duplicate tins of each treatment, together with the percentage infection for the sum of these numbers.

Table II.

Exp. 1. *Percentage of infection at the different temperatures for the four treatments.*

	Soil temperature						
	15° C.	19° C.	23° C.	27° C.	31° C.	35° C.	Average % for 5 temps.
Sterilised externally.							
I. No. of seedlings	6	6	10	9	9	10	0
No. infected	0	0	0	0	0	0	
II. No. of seedlings	8	8	10	10	10	10	
No. infected	0	0	0	0	0	0	
Average %	0	0	0	0	0	0	
Untreated.							
I. No. of seedlings	2	9	10	8	8	10	10·6
No. infected	0	2	1	3	1	0	
II. No. of seedlings	5	6	9	9	1	9	
No. infected	0	1	0	1	0	0	
Average %	0	20	5·2	23·5	6·7	0	
Inoculated plain.							
I. No. of seedlings	1	8	10	9	9	10	21·3
No. infected	?	2	0	3	2	2	
II. No. of seedlings	2	8	10	10	10	10	
No. infected	?	2	2	2	3	2	
Average %	?	25	10	26·3	26·3	20	
Inoculated chipped.							
I. No. of seedlings	0	6	8	6	7	7	72·3
No. infected	?	4	3	6	6	4	
II. No. of seedlings	1	3	8	5	7	8	
No. infected	?	3	6	5	4	6	
Average %	?	78	56	100	71·5	66·6	
Average % for three treatments where infection occurred	?	35	24	42·6	33·4	26	

As mentioned previously, the seeds at a soil temperature of 15° C. germinated very irregularly and made exceedingly poor growth. The only exception was in the case of the externally sterilised seed, of which six and eight respectively out of ten germinated. No infection was apparent on the seedlings which did appear, but in view of the fact that the sterilised seed germinated more or less normally, there is a strong presumption that the poor germination of the inoculated seed was due to infection. This cannot, however, be stated with certainty. In a later

experiment (see Exp. 2) severe infection was obtained at 15° C., and this lends support to the hypothesis. In view of this uncertainty as to the cause of the poor germination at this temperature, therefore, this column of figures has been omitted in calculating the average percentage infections for each treatment.

It will be seen from Table II that the most striking result is that due to variation in seed treatment. No infection in any case was found on the seedlings produced from seed which had been externally sterilised. Infection was low in the case of the untreated seed, but sufficiently high to show that, under the given set of air conditions, an appreciable amount of disease may develop. The presumption from these two results would appear to be that, for this batch of seed at least, disease arose only from organisms carried on the outside of the seed. Where the organism was present in much larger numbers ("inoculated plain") a greater amount of disease was produced, whilst where the organism was introduced actually within the seed-coat and hence in contact with the embryo from the beginning of germination, very severe infection resulted. In this connection a factor which cannot be shown numerically should be taken into account, namely, the severity of attack. In the case of the inoculated chipped seed the infection was very severe, often involving the entire cotyledon, and in some cases resulting in the death of the seedling. With the plain inoculated seed the attack of individual plants was less severe, but still very conspicuous. With the untreated seed, on the other hand, the infection was usually very much less in degree, often being limited to one or two spots on the edge of the cotyledon. The variation in degree of infection was thus more marked than the figures in Table II show.

With regard to the effect of soil temperature, it will be seen that, while there appears to be some influence, this is less marked than the difference between treatments. A marked falling off in degree of infection is apparent at the higher temperatures, and this is true for each treatment. Nevertheless, it is clear that even so high a soil temperature as 35° C. is insufficient to inhibit the development of the disease except on the untreated seed.

Exp. 2. The plan of this experiment was similar to that of Exp. 1. The same four treatments were applied, but in this case, in order to ascertain whether a still higher temperature would inhibit the disease, a wider range of soil temperatures was used, namely, 15° C., 20° C., 25° C., 30° C., 35° C., 40° C. The only other differences in the two experiments were that, in this case, in place of glasshouse compost, cotton-growing soil obtained from the Gezira Plain in the Sudan was used, and

that the air conditions were set at 27° C. and an average of 85 per cent. humidity.

The seed was sown slightly more deeply than in the previous case, and this accounts for the longer period before the appearance of the seedlings. Germination was good and rather more even than in the previous experiments. The numbers germinating out of eighty seeds sown are given in Table III.

Table III.

Percentage germination at different temperatures (Gezira soil).

	15° C.	20° C.	25° C.	30° C.	35° C.	40° C.
Time for germination (days)	14	8	5	5–6	5	7
Total no. germinated	37	53	60	55	42	29
Germination %	45·3	66·3	75·0	68·8	52·5	36·3

Table IV.

Exp. 2. Percentage of infection at the different temperatures for the four treatments.

	Soil temperature						
	15° C.	20° C.	25° C.	30° C.	35° C.	40° C.	Average % for 6 temps.
Sterilised externally.							
I. No. of seedlings	6	8	10	10	8	2	
No. infected	0	0	0	0	0	0	
II. No. of seedlings	10	9	10	10	9	7	0
No. infected	0	0	0	0	0	0	
Average %	0	0	0	0	0	0	
Untreated.							
I. No. of seedlings	1	7	8	8	5	4	
No. infected	0	5	6	3	1	0	
II. No. of seedlings	3	4	8	7	6	7	39·7
No. infected	2	0	5	3	2	0	
Average %	50·0	45·4	68·8	40·0	27·2	0	
Inoculated plain.							
I. No. of seedlings	5	5	9	5	6	2	
No. infected	4	2	6	5	3	0	
II. No. of seedlings	5	8	7	8	3	3	63·6
No. infected	4	8	4	3	2	1	
Average %	80·6	77·0	62·5	61·5	55·6	20·0	
Inoculated chipped.							
I. No. of seedlings	3	4	6	2	1	2	
No. infected	3	4	6	2	1	2	
II. No. of seedlings	4	8	2	4	2	1	92·4
No. infected	4	8	2	2	2	1	
Average %	100·8	100	100	67·7	100	100	
Average % for three treatments where infection occurred	81·0	75	72·5	53·0	47·8	21	

Infection was more severe than in the earlier experiment, and 2 weeks after sowing was apparently completely developed. Examination of the seedlings was made 17 days after sowing, except in the case of the seedlings at a soil temperature of 15° C. when a further 7 days was allowed for full development of infection owing to the delayed germination. The results are given in Table IV.

It will be seen that the results of this experiment fully bear out those of the previous trial. Again no infection was found on the externally sterilised seed at any temperature, while some infection occurred in all other cases except on the untreated seed at 40° C. The effect of temperature in this experiment is more marked, there being a steady fall in average infection with increasing temperature when the three treatments in which infection resulted are considered together. A brief discussion of the results of these two experiments is given later in this paper.

The influence of soil temperature on secondary infection.

In order to test whether soil temperature would have any influence on the susceptibility of the plant to external (secondary) infection and, at the same time, to endeavour to obtain confirmatory evidence for the theory of possible latency and spread of the organism within the plant, a further experiment was carried out on the plants in Exp. 1. The plants were allowed to grow on for a week after the examination for primary infection had been made, by which time they had reached a height of about 18 inches and had produced several true leaves. These newly formed leaves were entirely free from disease. Four tins, one of each seed treatment, were then removed from each tank and the plants thoroughly sprayed, by means of an atomiser, with a strong suspension of *B. malvacearum* in water. They were returned to the chambers, and allowed to remain under the same conditions as before, except that the relative humidity was increased to 85–90 per cent. to provide the best possible conditions for infection. The sprayed plants occupied the left half of each chamber, the four tins of plants in the right half being left unsprayed. After 18 days all the plants were examined for infection individually[1]. The amount of infection was very variable between different plants of the same tin, and an estimate of the degree of infection was difficult to obtain. Finally the procedure was adopted of grading the plants in three classes which are shown in columns 5, 6, and 7 of Table V. A plant showing a few scattered spots only was classed as "lightly infected"; up to fifteen lesions on a plant constituted "moderate

[1] This examination was made by Dr W. B. Brierley.

infection," while a greater amount of disease was estimated as "heavy infection."

Table V.

Influence of soil temperature on secondary infection.

Soil temp.	Treatment	No. of plants	Infection			
			None	Light	Moderate	Heavy
15° C.	Sterilised[1]	7	—	3	1	0
	Untreated	0	—	—	—	—
	Inoculated:					
	Plain	1	—	1	—	—
	Chipped	0	—	—	—	—
19° C.	Sterilised	5	0	4[2]	1[3]	0
	Untreated	8	2	2[4]	3	1
	Inoculated:					
	Plain	8	2	2	3	1
	Chipped	4	1	2	1[5]	0
23° C.	Sterilised[6]	9	2	4	1	0
	Untreated	10	1	6[7]	1	2
	Inoculated:					
	Plain	10	4	5	0	1
	Chipped	7	1	5	1	0
27° C.	Sterilised[8]	9	0	4	1	2
	Untreated	8	4	1	0	3
	Inoculated:					
	Plain	10	0	6	2	2
	Chipped	4	0	1	2	1
31° C.	Sterilised	4	0	3	0	1
	Untreated	5	0	2	2	1
	Inoculated:					
	Plain	7	0	3	3	1
	Chipped	6	0	4	1	1

[1] Plants very small with much reduced leaves.
[2] One plant with numerous doubtful lesions.
[3] Plus numerous doubtful lesions.
[4] One plant with numerous doubtful lesions.
[5] Plus numerous doubtful lesions.
[6] Two remaining plants with doubtful lesions.
[7] One plant with numerous doubtful lesions.
[8] Two remaining plants with doubtful heavy infection on one leaf of each.

On the basis outlined the infection at 15° C. appears to be very slight, but it should be borne in mind that there were only eight sprayed plants altogether, and that these were extremely small with only one or two very reduced leaves. Thus the infection in this case was relatively as severe as in any of the other chambers.

It will be seen from Table V that it is impossible to detect any marked effect in degree of infection due either to soil temperature or to previous seed treatment. There appears to be somewhat more infection at 27° C. soil temperature, but this is hardly significant. Infection was irregular but widespread and occurred at all soil temperatures.

The unsprayed plants showed no infection except in one or two cases where a leaf touched one of the sprayed plants, when infection was transmitted by contact to this leaf. The significance of this lack of infection will be discussed later.

Discussion of results and laboratory experiments.

Several points are apparent from a study of the results of the experiments described. With regard to the location of the parasite in the seed, the experiments on primary infection show that, although the seed was derived from diseased plants, thorough external disinfection of the seed results in the production of healthy seedlings showing no sign of the disease. This would appear to indicate that, for these batches of seed at least, the organism was not present in a virulent state within the seed. It might, however, be argued that the treatment with sulphuric acid had stimulated germination (a fact known to be the case) and thus enabled the seedlings to resist infection. That this is the explanation is not borne out, however, by laboratory experiments on this same seed. Samples were taken and subjected to a more vigorous sterilisation than can be achieved by sulphuric acid and mercuric chloride alone. All cotton-seed bears at the micropylar end a very close tuft of short fuzz which is difficult to wet, and is not completely removed even by 15 minutes' treatment with sulphuric acid. In the tests to be described this tuft was removed by scraping each seed individually with a scalpel under a dissecting microscope until the exterior presented a clean surface free from any hairs. The seed was then sterilised in 1 : 500 mercuric chloride under the vacuum pump, washed in sterile water and crushed in sterile broth. Parallel samples were first dehusked, the embryo extracted and lightly sterilised externally and similarly crushed in broth. Platings from these samples showed no colonies of *B. malvacearum* in either case, the dehusked seed producing no organisms whatever, while the former samples gave colonies of a number of different saprophytic bacteria and fungi. Control samples similarly sterilised, but not crushed, produced no colonies. The organisms isolated in the case of the crushed seed had, therefore, apparently originated from a position between the seed-coat and the embryo, or possibly within the micropylar passage.

Taking these results into consideration, it is reasonable to conclude that the seed used in this experiment did not contain the organism within it, although the seed was derived from heavily infected plants in the Sudan. Some infection occurred, however, on the seedlings from untreated seed, and hence the conclusion is reached that the organism was

present on the exterior of the seed or in the fuzz. The heavy infection resulting from inoculation of the outside of the seed shows that presence of the parasite in this position is sufficient to account for severe damage. It would appear that more extensive trials in the field with various methods of external disinfection of seed might be well worth while.

So far as the effect of soil temperature is concerned, the experiments described do not entirely accord with the results given by Massey. Infection can occur at all soil temperatures at which growth of the plant is possible, the air temperature being 25°–27° C. A falling-off in amount is noticeable at temperatures above 30° C., but this is not sufficient in degree to render it likely that sowing of seed at the time of high soil temperature will result in effective control. Low temperatures, on the other hand, appear to increase the amount of infection, even when carried below a temperature at which the plant can make reasonable growth.

Considering now the experiments on secondary infection, it is seen that neither soil temperature nor seed treatment has had any marked influence on infection resulting from spray inoculation. This result is hardly surprising, since the attack of a local parasite on the purely aerial parts of the plant could only be indirectly affected by soil temperature through the effect of this factor on the metabolism and growth of the host. If, however, the theory of possible latency and internal spread of the organism were correct, it would be expected that the unsprayed plants which had been infected in the cotyledonary stage would show further infection when placed under these conditions demonstrably suitable for development of infection. That this was not so indicates that under these conditions at least internal spread had not taken place.

The accident that a few leaves of the unsprayed plants touched some of the leaves of the sprayed and became infected at these points confirms the view that infection is easily transmitted by contact under humid conditions.

Summary.

Experiments on the angular leaf-spot disease of cotton carried out under controlled conditions lead to the following conclusions:

1. Seed derived from diseased plants may give rise to infected seedlings.

2. This infection is due to bacteria carried on the outside of the seed and in the fuzz.

3. Thorough disinfection of the exterior of the seed results in healthy seedlings.

4. The amount of primary infection resulting from infected seed decreases at soil temperatures above 30° C., but infection is not inhibited at 40° C.

5. Soil temperature has little or no effect on secondary infection resulting from spray inoculation of the plants.

6. Plants diseased in the seedling stage grow out free from disease, if no further inoculation occurs.

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THE EFFECT OF CERTAIN TREATMENTS ON THE GERMINATION OF TOMATO SEEDS[1]

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Introduction.

The disinfection of seeds by chemical means is now an established process in the control of many seed-borne diseases, and there is no need to discuss the extensive literature on this subject. The experiments described below were started to determine how different treatments affect the germination of tomato seeds.

Methods.

Air-dry seeds collected in 1928 were used, the varieties being E.S. 1 and E.S. 2—two tomato strains raised at the above institute. The batch of E.S. 1 seed showed an exceptionally low percentage germination, and served to indicate the effect of chemical treatment on weak seeds. Two batches of E.S. 2 seeds were used and designated E.S. 2 and E.S. 2_1 respectively. The tests were conducted at different times between January and June, and a comparison between series is hardly admissible.

Seeds were sown in the usual shallow boxes filled with baked cucumber soil. Each box was divided into two parts and contained two lots of 50 seeds treated differently prior to sowing. Each seed treatment was repeated in two different boxes. In some cases the sowing was repeated on moist filter paper in Petri dishes. No exact temperature records were taken in the glasshouse although, when possible, the night minima and day maxima were noted, as well as the temperature three times a day, and an approximate mean day temperature was calculated for each germination period. The most interesting results are presented in tables as actual numbers of germinated seeds at 2-day intervals, and as the total percentage germination on the 20th day after sowing.

To estimate the differences in total percentage germination, resulting from different treatments, all results are expressed as a percentage of the corresponding controls. A difference is considered significant when it

[1] Work done when the writer was staying in England under an allowance from the Polish Government.

exceeds 10 per cent. of the control for E.S. 1 and 3 per cent. for both lots of E.S. 2. These limits are obviously conditional, and are calculated on the basis of the greatest difference between a separate control and the arithmetic mean of all controls belonging to the same lot of seeds.

A. TREATMENT WITH FUNGICIDES.

The following compounds were used as solutions in tap water:

(1) Formaldehyde (38–40 per cent. commercial formaldehyde).

(2) Hydrogen peroxide (20 vols.).

(3) Mercuric chloride.

(4) Copper sulphate.

The seeds were treated in flasks at laboratory temperature (15–18° C.) and an excess of the liquid was always used (about 20 c.c. for every 100 seeds), the flasks being well shaken during the period of treatment to secure satisfactory wetting of the hairy seeds. The seeds were afterwards strained and either dried on blotting paper or sown wet immediately after treatment.

(1) *Formaldehyde treatment.*

Tables I and II show some of the results obtained by treating seeds with formaldehyde solutions.

It will be seen that in the case of weak seeds, while concentrations up to 1 per cent. formaldehyde were without effect, those of 2 and 5 per cent. markedly decreased the percentage germination. The increased germination for 5 per cent. formaldehyde on the 24th day suggests that a longer germination period would have changed the final figures, and that the effect of treatment is largely retardation of germination.

There was a striking difference between seeds sown wet and those sown after drying. In the latter case even 0·1 per cent. formaldehyde proved injurious, probably due to the concentration of formaldehyde around the seed as the result of drying. The injurious effect of formaldehyde in this series was no doubt due to the nature of the E.S. 1 seeds, for, as may be seen from Table II, the same treatments, applied to seeds of a higher germination power, proved harmless.

Table II represents results where strong seeds were dried for different periods after treatment. Ten minutes' treatment with all the concentrations tested merely retarded the germination rate, but 2 per cent. formaldehyde seemed injurious in 15-minute exposures.

An attempt was made to correct the injurious effect of formaldehyde by various after-treatments, such as drying for 2 hours at 15° C. or

Table I.

The effect of formaldehyde treatments on the germination of tomato seeds. Variety E.S. 1. Significant differences in total percentage germination 7·10 per cent. of the controls.

Time of exposure and strength of formaldehyde solution	Sowing in baked soil	No. of seeds germinated, day after sowing: 8th	10th	12th	14th	16th	18th	20th	Total % germination on 20th day	Germination in % of controls	Results of additional countings
5 min.	Wet										
Tap water control	,,	24	12	2	1	—	—	—	78	100·0	—
5 %	,,	—	2	1	3	6	3	3	36	46·2	54 % on 24th day
2	,,	—	7	7	7	4	3	1	58	74·5	No additional records taken
1	,,	16	15	4	2	2	1	—	80	102·5	,,
0·5	,,	7	18	9	2	2	1	1	80	102·5	,,
0·1	,,	7	22	7	—	—	—	—	72	92·4	,,
	Dried at 15° C. 15 hr.										
Tap water control	,,	9	26	3	—	1	—	—	78	100·0	,,
5 %	,,	—	—	—	—	—	—	—	—	—	6 % on 30th day
2	,,	—	—	—	—	—	1	2	6	7·7	32 % on 30th day
1	,,	—	1	3	4	3	11	1	46	59·0	66 % on 30th day
0·5	,,	—	6	7	6	2	3	1	50	64·2	No additional records taken
0·1	,,	6	19	3	2	2	—	1	66	84·6	,,
10 min.											
Tap water control	,,	3	51	11	7	1	4	—	77	100·0	
5 %	,,	—	—	—	—	—	—	—	—	—	1 % on 28th day
2	,,	—	—	—	—	—	—	—	—	—	2 % on 28th day
1	,,	—	—	—	1	2	—	—	3	3·0	20 % on 28th day
0·5	,,	—	1	3	8	11	12	1	36	46·8	No additional records taken
0·1	,,	—	20	25	20	5	2	3	75	97·5	,,

Note. The mean day temperature was 15·5° C., the night minimum 8·5° C. and day maximum 25·5° C.

Table II.

The effect of different periods of drying on germination of tomato seeds treated with formaldehyde. Variety E.S. 2_1. Significant differences in total percentage germination 3 per cent. of the controls.

Time of exposure and strength of formaldehyde solution	Time of sowing after treatment	No. of seeds germinated, day after sowing: 6th	8th	10th	12th	14th	16th	18th	20th	Total % germination on 20th day	Total germination in % of controls	Germination on 12th day in % of total germination
5 min.												
Untreated control	30 min.	—	15	53	29	—	—	—	—	97	100·0	100·0
5 %	,,	—	10	41	35	10	2	1	—	99	102·0	87·0
2	,,	1	13	48	22	8	1	2	2	97	100·0	86·6
1	,,	—	14	45	30	7	—	—	1	97	100·0	91·7
5	1 hr.	—	—	7	42	21	16	4	3	93	96·0	52·7
2	,,	—	8	31	44	10	—	1	1	95	98·0	87·5
1	,,	—	9	61	22	3	1	—	—	96	99·0	95·8
5	17 hr.	—	2	25	41	12	10	3	2	95	98·0	71·6
2	,,	—	12	47	29	2	4	2	—	96	99·0	91·6
1	,,	—	18	44	28	5	—	1	—	96	99·0	93·8
10 min.												
Untreated control	17 hr.	—	31	61	2	1	1	—	—	96	100·0	98·0
5 %	1 hr.	—	24	54	15	3	1	1	—	98	102·0	95·0
2	,,	—	38	47	3	3	1	—	1	93	97·0	94·7
1	,,	1	37	46	7	4	1	1	—	97	101·0	94·0
5	5½ hr.	—	10	49	19	8	6	—	3	95	99·0	82·0
2	,,	—	18	51	13	7	2	—	2	93	97·0	88·2
1	,,	—	25	52	10	7	2	2	—	98	102·0	88·7
15 min.												
Untreated control	5½ hr.	—	31	61	2	1	1	—	—	96	100·0	98·0
5 %	,,	—	—	1	2	1	2	5	4	15	15·6	84 % on 34th day
2	,,	—	5	23	16	9	17	7	8	85	88·5	No additional records taken
1	,,	1	33	43	8	2	2	1	2	92	96·0	92·5

Note. The mean day temperature was 23° C., the minimum night temperature 8° C. and maximum day temperature 42° C.

Table III.

The effect of mercuric chloride treatments on germination of tomato seeds.

Variety E.S. 1. *Significant differences in total percentage* 7·10 *per cent. of controls.*

Time of exposure and strength of solution	Sowing in baked soil	No. of seeds germinated, day after sowing: 8th	10th	12th	14th	16th	18th	20th	Total % germination 20th day	Germination in % of controls
10 min.	17 hr. dried at 15–18° C.									
Tap water control	,,	23	52	3	3	2	—	—	83	100·0
1 % mercuric chloride	,,	—	—	—	—	—	—	—	—	—
0·5 ,, ,,	,,	—	—	—	1	4	2	2	9	10·8
0·1 ,, ,,	,,	1	5	14	12	18	8	5	61	73·5
0·05 ,, ,,	,,	8	47	16	5	2	1	—	79	95·2
15 min.										
Tap water control	,,	8	50	10	3	1	1	2	75	100·0
1 % mercuric chloride	,,	—	—	—	—	—	—	—	—	—
0·5 ,, ,,	,,	—	—	—	—	1	—	—	1	1·3
0·1 ,, ,,	,,	1	13	28	15	15	5	—	77	102·0
0·05 ,, ,,	,,	—	22	36	16	4	2	1	81	107·5

Variety E.S. 2_1. *Significant differences in total percentage* 3 *per cent. of controls.*

Time of exposure and strength of solution	Sowing in baked soil	8th	10th	12th	14th	16th	18th	20th	Total % germination 20th day	Germination in % of controls
15 min.	19 hr. dried at 16·5° C.									
Tap water control	,,	3	36	34	14	7	3	—	97	100·0
1 % mercuric chloride	,,	—	—	—	—	—	—	—	—	—(*a*)
0·5 ,, ,,	,,	—	—	—	1	1	1	—	3	3·1
0·1 ,, ,,	,,	—	—	14	10	26	18	4	72	74·2(*b*)
0·05 ,, ,,	,,	1	4	22	[illegible]	27	11	10	84	86·6(*c*)

Note. The mean day temperature was 15·5° C., the night minimum 8·5° C. and the day maximum 25·5° C.
(*a*) No increase on 28th day; (*b*) 89 % on 28th day; (*c*) 96% on 28th day.

30 minutes at 35° C., washing treated seeds with either water or ammonia and sowing them after drying at 15° C. The effects of these after-treatments could not be stated definitely as the pre-treatment (soaking seeds for 5 minutes in a 2 per cent. solution of formaldehyde) applied to E.S. 2 seeds of a high germination power did not diminish their vitality.

(2) *Hydrogen peroxide treatment.*

Solutions between 100 and 1 per cent. of hydrogen peroxide, 20 vols., applied for 10 or 30 minutes to seeds of the variety E.S. 1, resulted chiefly in a retardation of germination, most pronounced with the strongest solutions.

(3) *Mercuric chloride treatment.*

Table III shows the results obtained by treating seeds with mercuric chloride solutions.

In all cases germination was retarded, and only the 0·05 per cent. solution failed to affect the vitality of the seeds. The 0·1 per cent. solution was only slightly injurious, but those of 0·5 per cent. and above were markedly so. Germination failed in Petri dishes, probably owing to the persistence of mercuric chloride, as the seeds were not washed after treatment.

(4) *Copper sulphate treatment.*

Table IV represents some of the results obtained.

Table IV.

The effect of copper sulphate treatments on germination of tomato seeds. Variety E.S. 2_1. *Significant differences in total percentage germination 3 per cent. of controls.*

Treatment: Strength of solution	Time of exposure	No. of seeds germinated, day after sowing: 8th	10th	12th	14th	16th	18th	20th	Total % germination on 20th day	Germination in % of controls
Untreated control		4	29	35	28	1	—	1	98	100·0
10 % copper sulphate	10 min.	1	6	8	45	19	4	6	89	90·8(*a*)
5 ,, ,,	20 ,,	—	10	17	50	10	5	3	95	97·0
2 ,, ,,	50 ,,	—	—	16	52	16	7	5	96	98·0
1 ,, ,,	100 ,,	3	9	16	43	9	3	1	84	85·7(*b*)
0·5 ,, ,,	200 ,,	1	9	23	49	10	—	2	94	96·0(*c*)

Note. The mean day temperature was 23·5° C., the night minimum 6·5° C. and the day maximum 40° C. The treated seeds were sown in baked soil 24 hr. after treatment; dried at 15–18° C.

(*a*) 94 % on 28th day; (*b*) 94 % on 28th day; (*c*) 96 % on 28th day.

The retarding effect of all treatments is evident, but the percentage germination was hardly affected.

In two other series it has been found that copper sulphate solutions between 10 per cent. and 0·5 per cent. applied for 10 minutes and 15 minutes slightly retarded the germination of seeds sown 24 hours after treatment. The decreases in total percentage germination, if any, were too small to be considered. Tests in Petri dishes confirmed the results obtained in boxes.

B. Soaking seeds in water of different temperatures.

(*The "wet heat" treatment.*)

Tomato seeds, E.S. 2 and E.S. 2_1 in muslin bags, were soaked in water for periods varying from 10 minutes to 15 hours over a temperature range of 15° C. to 60° C. After treatment they were sown immediately in baked soil. Control seeds were sown without any previous soaking. The results are given below.

Table V indicates that soaking in water at 15° C. hastened germination slightly, the effect being most pronounced after 6 hours' treatment. Some retardation occurred at 35° C. except when the period of exposure was 15 hours, which suggests that the beneficial effect of soaking counteracted to some extent the effect of the high temperature. A similar result was obtained at 40° C., 3 hours' soaking giving the best result, while at 46° C. germination was hastened by soaking for 10 minutes, and retarded by longer exposures. Soaking in water at 50·5° C. reduced the rate of germination still further, and the final treatments at 56° C. and 60·5° C. were distinctly injurious.

C. Drying seeds at different temperatures.

(*The "dry heat" treatment.*)

Tomato seeds were heated for different periods in an electric oven, the results being given in Table VI. Temperatures of 30°–50° C. for 72 hours did not alter the germination, and are not discussed further.

Table VI shows that air-dried tomato seeds can withstand dry heat up to 79·5° C. even for 72 hours, the germination being more or less retarded, according to the temperature and time of exposure. 90° C. and 100° C. proved very injurious, although, in some cases, the seeds still retained a fairly high germination power. It should be mentioned that a large percentage of seedlings from seeds heated at 85° C., 90° C. and 100° C. died soon after emergence. The survivors grew very slowly and developed abnormally.

Table V.

The effect of soaking in hot water on the germination of tomato seeds. Variety E.S. 2. Significant differences in total percentage germination 3 per cent. of controls.

Time of soaking and temperature of water	Number of seeds germinated, day after sowing: 6th	8th	10th	12th	14th	16th	18th	20th	Total % germination on 20th day	Germination in % of the controls
15° C.										
Untreated control	1	20	35	37	6	—	1	—	100	100
3 hr.	—	15	58	24	3	—	—	—	100	100
6 ,,	—	20	71	6	2	—	—	—	99	99
15 ,,	2	27	45	22	1	1	—	—	98	98
35° C.										
Untreated control	—	3	61	30	3	1	—	—	98	100
3 hr.	—	—	45	37	10	6	—	—	98	100
6 ,,	—	—	34	56	6	3	—	—	99	100·9
15 ,, *	—	—	71	24	2	2	—	—	99	100·9
40° C.										
Untreated control	—	72	23	2	2	1	—	—	100	100
1 hr.	—	38	54	6	1	—	—	—	99	99
3 ,,	—	82	13	1	1	—	—	—	97	97
6 ,,	—	59	36	3	1	1	—	—	100	100
15 ,, †	—	52	41	5	1	—	—	—	99	99
46° C.										
Untreated control	—	27	71	2	2	2	—	—	99	100
10 min.	4	74	17	3	—	—	—	—	98	99
30 ,,	—	31	65	3	—	—	—	—	99	100
1 hr.	1	36	58	1	2	—	—	—	98	99
3 ,,	—	4	84	9	—	1	1	—	99	100
6 ,,	—	1	74	21	3	—	—	1	100	100·8
15 ,,	2	18	48	19	4	2	4	1	98	99
50·5° C.										
Untreated control	—	28	53	13	3	—	1	—	98	100
10 min.	—	5	85	7	1	—	—	—	98	100
30 ,,	—	5	73	16	2	4	—	—	100	101·8
1 hr.	—	1	74	16	3	—	1	2	97	99
3 ,,	—	—	18	38	29	6	1	2	94	96
6 ,,	—	—	1	11	38	28	11	7	96	98
15 ,,	—	—	—	1	—	3	14	15	32	32·7 (*a*)
56° C.										
Untreated control	—	20	35	22	9	3	3	2	94	100
10 min.	—	7	8	5	4	1	9	1	35	37·3 (*b*)
30 ,,	—	3	3	4	4	1	4	—	19	20·2 (*c*)
1 hr.	—	—	2	—	1	—	—	1	4	4·3 (*d*)
3 ,,	—	—	—	—	—	—	—	—	—	—
6 ,,	—	—	—	—	—	—	—	—	—	—
60·5° C.										
Untreated control	—	16	43	20	8	5	3	—	95	100
10 min.	—	—	2	3	7	2	6	2	22	23·2 (*e*)
30 ,,	—	—	—	—	—	—	—	—	—	—
1 hr.	—	—	—	—	—	—	—	—	—	—

Note. The mean day temperature was 23·5° C., the night minimum 5·5° C. and the day maximum 42° C.

* The temperature fell overnight to 30° C. † Temperature fell overnight to 35° C.

The following are percentage germinations after periods exceeding 20 days:
(*a*) 85 per cent. on the 31st day; (*b*) 50 per cent. on the 32nd day; (*c*) 29 per cent. on the 32nd day; (*d*) 8 per cent. on the 23rd day; (*e*) no further increase till the 31st day.

Table VI.

The effect of dry heat on germination of tomato seeds. Variety E.S. 2. Significant differences in total percentage germination 3 per cent. of controls.

Temperature and time of exposure	No. of seeds germinated, day after sowing: 6th	8th	10th	12th	14th	16th	18th	20th	Total % germination on 20th day	Germination in % of controls	Results of additional countings
75° C.											
Untreated control	—	2	19	43	18	14	2	1	99	100	
24 hr.	—	—	10	19	21	27	2	7	86	87	28th day 95 %
48 „	—	—	6	4	13	17	11	25	76	76·8	„ 91
72 „	—	—	1	10	8	21	4	19	63	63·6	„ 89
79·5° C.											
Untreated control	—	3	24	28	27	14	1	1	98	100	
3 hr.	—	—	11	23	25	28	3	3	93	95	28th day 95 %
6 „	—	—	6	21	31	28	3	2	91	92·9	„ 96
24 „	—	—	—	5	18	26	8	17	74	75·5	„ 89
48 „	—	—	—	—	9	20	14	22	65	66·3	„ 88
72 „	—	—	—	1	12	17	12	14	56	57·2	„ 84
85° C.											
Untreated control	6	38	23	14	7	2	6	2	98	100	
10 min.	—	—	5	8	5	6	10	10	44	44·9	35th day 62 %
30 „	1	3	17	16	6	8	23	5	79	80·6	„ 87
1 hr.	—	6	15	12	27	9	11	9	89	90·8	„ 93
3 „	—	—	3	7	2	4	12	9	37	37·8	„ 62
6 „	—	1	10	5	16	1	13	7	53	54·1	„ 64
90° C.											
Untreated control	—	17	30	42	—	—	6	2	97	100	
10 min.	—	—	—	7	—	—	26	7	40	41·3	47th day 68 %
30 „	—	—	—	1	—	—	13	8	22	22·7	„ 64
1 hr.	—	—	—	—	—	—	8	8	16	16·5	„ 37
3 „	—	—	—	—	—	—	1	2	3	3·1	„ 21
6 „	—	—	—	—	—	—	1	—	1	1·0	„ 8
100° C.											
Untreated control	—	15	49	21	8	—	6	—	99	100	
10 min.	—	—	—	—	3	—	1	9	13	13·3	47th day 33 %
30 „	—	—	—	1	—	—	1	3	5	5·1	„ 17
1 hr.	—	—	—	—	—	—	1	—	1	1·0	„ 10
3 „	—	—	—	—	—	—	—	—	—	—	
6 „	—	—	—	—	—	—	—	—	—	—	

Note. The mean day temperature was 25° C., the night minimum 11° C. and the day maximum 42° C.

The writer is much indebted to Dr W. F. Bewley, Director of the Cheshunt Experimental and Research Station, who suggested these experiments.

SUMMARY.

1. The effect of soaking tomato seeds in certain compounds and of exposing them to varying periods of wet and dry heat has been investigated.

2. Good seeds (germination power 98–99 per cent.) withstood solutions of formaldehyde, not stronger than 2 per cent. (1 part per volume 38–40 per cent. commercial formaldehyde to 19 parts water) for 10 minutes, and not stronger than 1 per cent. for 15 minutes, followed by drying at 15–18° C. A 5 per cent. solution for 10 minutes, which was without effect when seeds were sown wet, retarded the germination after drying but did not affect the seeds' vitality.

3. Seeds of an inferior quality (80 per cent. germination) withstood solutions of formaldehyde not stronger than 1 per cent. for 5 minutes when sown wet, and were affected even by 0·1 per cent. formaldehyde for 5 minutes when dried after treatment.

4. 100, 50, 35, 10, 5 and 1 per cent. solutions of hydrogen peroxide (commercial 20 vols. hydrogen peroxide) for 10 and 30 minutes, followed by drying, retarded germination, which was more pronounced with stronger solutions, the vitality of the seeds being practically unaffected.

5. An 0·05 per cent. mercuric chloride solution for 10 minutes proved harmless, but higher concentrations and longer treatment either checked the germination of dried seeds or retarded it markedly.

6. The vitality of seeds was not affected by copper sulphate solutions as follows: 10 per cent. for 15 minutes, 5 per cent. for 20 minutes, 2 per cent. for 50 minutes, 1 per cent. for 100 minutes and 0·5 per cent. for 200 minutes, but the germination in some cases was markedly retarded. A 5 per cent. solution of copper suphate for 10 minutes proved practically harmless.

7. Good air-dried seeds withstood hot water at 35–46° C. for 15 hours and at 50·5° C. for 1 hour without any important injury. Higher temperatures decreased the percentage germination markedly even after 10 minutes' soaking.

8. Air-dry seeds were not affected by exposure to dry heat of 30–50° C. for 72 hours. They withstood dry heat up to 79·5° C. even for 72 hours, the germination, however, being more retarded as the temperature and time of exposure increased. Temperatures above 79·5° C. proved very injurious at 10 minutes' exposure.

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SOME OBSERVATIONS ON TOMATO PLANTS FROM SEED SUBMITTED TO HIGH TEMPERATURES[1]

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(With 4 Text-figures.)

DURING an investigation of the effect of heating tomato seeds (2) attention was drawn to the abnormal appearance of the seedlings from seeds heated at temperatures between 85° C. and 100° C. Their growth was abnormally slow, the hypocotyls were short and thick, and the cotyledons were fleshy and distorted. Usually one cotyledon was more affected than the other, and frequently only one developed. The plumule failed to produce a normal shoot, being represented by a callus-like swelling. A high percentage of the seedlings, especially those from seeds heated at 90° C. and

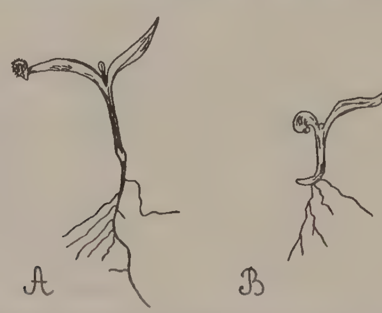

Fig. 1. Tomato seedlings (nat. size): *A*, normal; *B*, abnormal.

100° C., died soon after emergence, roots being absent in every case. In those surviving the primary root was absent, but a few secondary roots had developed. Fig. 1 shows a normal (*A*) and abnormal (*B*) seedling.

A number of the abnormal seedlings were potted for better observation. Some developed only a few fleshy dark green leaves, frequently misshapen and curled, like plants shown in Fig. 2. Amongst these was one which developed a normal shoot on a distorted leaf (Fig. 2, *E*). Some of the seedlings although abnormal when potted became normal later,

[1] Work done in 1929 when the writer was staying in England under an allowance from the Polish Government.

an apparently normal shoot having developed from the swelling formed in place of the plumule (Fig. 3, *D*, *E*, *F*, *G*). In some cases more than one new shoot developed. Occasional individuals were apparently quite normal (Fig. 3, *H*). The observations could not be continued to the final stages of growth, and no data are available concerning flowering and fruiting of the abnormal plants. In further trials, described below, an

Fig. 2. Tomato plants raised from heated seeds. *E*, developed a normal shoot, *a*. (Nat. size.)

attempt was made to counteract the injurious effects of high temperatures by pre-drying the seeds at lower temperatures.

Commercial seeds of the variety Tuckswood were heated for varying periods at lower temperatures and then exposed for 1 hour either to 90° C. or to 88° C. Treated seeds were germinated on moist filter paper in Petri dishes, some of the sprouted seeds being transferred to pots of soil later to observe subsequent development. Germination occurred partly in darkness at 20–22° C., and partly in light at laboratory tempera-

ture, approximately 18° C. The heated seeds germinated more or less abnormally. The primary roots failed and the seedlings were twisted and distorted as shown in Fig. 4. In a few days new roots pushed through at the base, usually on the convex side (Fig. 4, *k*, *l*, *m*). When new rootlets replaced the injured primary root the seedling assumed a fairly normal

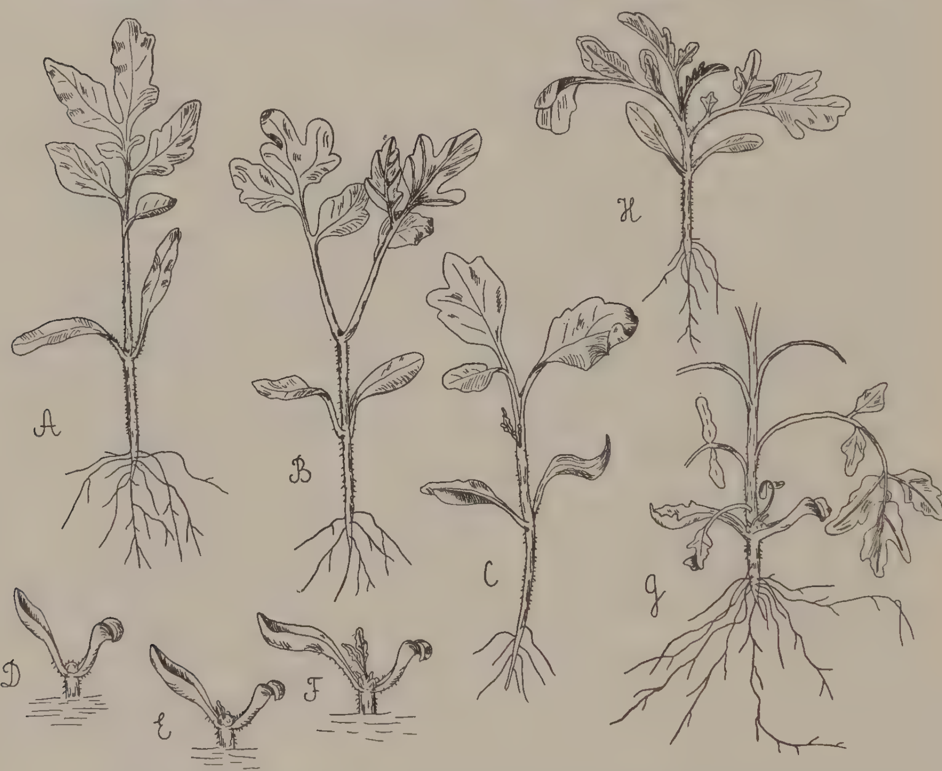

Fig. 3. Tomato plants from heated seeds. *D*, *E*, *F* and *G*, four successive developmental stages of a plant from seed heated at 85° C. for 10 minutes. *H*, apparently normal plant from heated seed. (Nat. size.)

appearance (Fig. 4, *n*, *t*). A proportion of the seedlings, probably those most severely injured, failed to develop new roots and soon decayed.

Distortion of seedlings was due to the tissue being injured on one side of the hypocotyl. Such injury was indicated by patches or stripes of white tissue. The affected regions, in many cases visible to the naked eye, always appeared on the concave side of the embryo, extending from the injured root end up to the plumule and further along the innermost cotyledon. The latter remained more misshapen than the outer cotyledon,

and sometimes failed to develop. The injured tissue fell away in pieces, during the course of further development, leaving deep wounds (Fig. 4, *o*, *p*). The latter healed gradually, and in severe cases distinct scars could be detected along the hypocotyl and on the cotyledons.

Seedlings which germinated late pushed their cotyledons through the seed-coats first (Fig. 4, *r*, *s*). Most seedlings which germinated abnormally,

Fig. 4. *a, b, c:* three successive germination stages of an untreated seed. ($\frac{3}{1}$ nat. size.)
d–n, q–r, s: heated seeds germinating abnormally; *q* of the same age as *c*. ($\frac{3}{1}$ nat. size.)
o: base of an abnormal seedling showing detachment of the injured tissue. ($\frac{6}{1}$ nat. size.)
p: part of the hypocotyl of an abnormal seedling showing pieces of injured tissue in the longitudinal wound. ($\frac{6}{1}$ nat. size.)
t: base of an apparently normally germinating seedling. ($\frac{6}{1}$ nat. size.)
u: embryo from an untreated seed. ($\frac{6}{1}$ nat. size.)
v: embryos from heated seed showing callus-like granulation in different parts. ($\frac{6}{1}$ nat. size.)
w: heated embryo which commenced growth inside the seed coats. ($\frac{6}{1}$ nat. size.)
z: seedling from a heated seed. ($\frac{6}{1}$ nat. size.)

and also some which appeared normal developed into abnormal plants. The germination records and the grouping of seedlings obtained from the various treatments are given in Table I.

The injurious effects of high temperatures were reduced by previously drying at lower temperatures, but germination was still retarded.

In Series I the mortality of the seedlings in Group A was greater than

Table I A.

Showing the effect of pre-drying tomato seeds prior to heating at high temperatures.

Series	No. of seeds	Pre-heating	Final heating	Sowing	% germination: Darkness at 20–22° C.: 3rd day	14th day	Darkness at 20–22° C. at night Laboratory 18° C. at day: 35th day	56th day	% seeds normal germina tion	No. of seed-lings potted	% sur-vival after 7 weeks
I. A	400	None	90° C. 1 hr.	Immediately	—	1·0	21·5	—	—	26	73·0
I. B	400	60° C. 20 hr. 18° C. 4 „ 71–72° C. 21 „ 18° C. 29 „	90° C. 1 hr.	„	0·5	18·5	86·0	—	—	78	94·0
II. 1	400	10–50° C. 11 hr. 18° C. 8½ „ 70° C. 19 „ 18° C. 3 „ 88° C. 1 „	88–102° C. 1 hr.	After 19 hr. at 18° C.	—	15·5	76·5	81·0	—	—	—
II. 2	400	—	88–102° C. 1 hr.	„	—	—	—	—	—	—	—
II. 3	400	10–50° C. 11 hr. 50–70° C. 27½ „ 70–88° C. 4 „	88–102° C. 1 hr.	After 19 hr. temp. falling 102° C. to 10° C.	—	26·0	81·8	86·5	—	—	—
Control	400	—	—	—	84·0	90·0	91·0	—	100	—	—
„	400	—	—	—	74·0	84·0	97·5	98·5	100	—	—

Table I B.

Series	Pre-heating	Final heating	Sowing	Grouping of seedlings about 4 weeks after potting: Seeds germinating normally				Seeds germinating abnormally				Total % of normal seedlings
						Abnormal plants %				Abnormal plants %		
				No. of plants	Normal plants	1st degree	2nd degree	No. of plants	Normal plants	1st degree*	2nd degree*	
I. A	None	90° C. 1 hr.	Immediately	—	—	—	—	19	—	47·0	53·0	—
I. B	60° C. 20 hr. 18° C. 4 ,, 71–72° C. 21 ,, 18° C. 29 ,,	90° C. 1 hr.	—	—	—	—	—	73	—	47·0	53·0	—
II. 1	10–50° C. 11 hr. 18° C. 8½ ,, 70° C. 19 ,, 18° C. 3 ,, 88° C. 1 ,,	88–102° C. 1 hr.	After 19 hr. at 18° C.	45	13·0	53·5	33·5	32	—	22·0	78·0	3·5
II. 3	10–50° C. 11 hr. 50–70° C. 27½ ,, 70–88° C. 4 ,,	88–102° C. 1 hr.	After 19 hr. temp. falling 102° C. to 10° C.	42	78·5	21·5	—	32	40·5	59·5	—	51·5

* 1st degree: cotyledons slightly injured; apical growing point injured; new shoots developing. 2nd degree: cotyledons badly injured; apical growing point injured; shoots absent.

in Group B, and the abnormality of the survivors more pronounced. The percentage of plants with a distinct scar along the hypocotyl, taken to indicate the degree of injury, was also higher for treatment A.

In Series II treatments 1 and 3 both gave a fairly high percentage germination, the longer period of pre-drying proving most effective.

The fairly high percentage of normal plants raised from abnormally germinating seeds (3) suggests that the basal growing point of a tomato embryo is least resistant to heat. The development of abnormal plants from apparently normal seedlings may be explained by replacement of the injured primary root by secondary organs which produced a more or less normal appearance. The abnormality of those seedlings could hardly be detected. The next parts attacked were the apical growing point, the hypocotyl, the inner and, lastly, the outer cotyledon.

Seeds which failed to germinate were dissected to examine the embryos. A number were decayed, but some seemed sound and had commenced to grow within the seed-coats. They possessed green cotyledons and hypocotyls, the root ends only having retained the white colour of a dormant embryo (Fig. 4, *w*). In some cases only parts of the embryonal tissue seemed to have retained their visibility, callus-like granulations being formed in those places, chlorophyll being present (Fig. 3, *v*). A number of embryos resembled those from untreated seeds (Fig. 4, *u*).

A similar abnormal germination was mentioned by Tapke (3) in wheat treated with hot water, and a very interesting description of abnormalities in *Helianthus annus* caused by high temperatures is given by Gain (1).

The writer is much indebted to Dr W. F. Bewley, Director of the Cheshunt Experimental and Research Station.

SUMMARY.

1. The results of pre-drying seeds prior to heating at high temperatures and a description of abnormalities in tomato seedlings caused by heat are given.

2. Commercial tomato seeds withstood 90° C. and 88–100° C. for 1 hour better when previously dried at lower temperatures, their germination being, however, markedly retarded.

3. Part of the heated seeds germinated abnormally, and the developing plants were more or less abnormal. The primary root did not develop, the cotyledons were misshapen, and the plumule failed to develop into a normal shoot.

4. The longer the period of pre-drying, the higher the rate of germination, and the less the degree of abnormality.

5. The basal growing point of a tomato embryo is least resistant to heat, the apical growing point, the hypocotyl, the inner and, lastly, the outer cotyledons following in order of resistance.

6. Embryos unable to push through the seed-coats can grow for a certain period within them and develop chlorophyll.

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A BOTANICAL STUDY OF PASTURE PLOTS

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CONTENTS.

Introduction.

In the year 1923 several seeds mixtures (mostly of the Cockle Park type) were laid down in large plots in a field on the Farm at Seale-Hayne Agricultural College, Devonshire. At the same time small plots of the door-mat type were sown down in the Botanical ground which were exact replicas of the larger plots. A few other mixtures were sown in similar plots—16*b*, 17*a*, and 17*b*—for comparison. Plot 6*b* was a blend of a Cockle Park and Elliot Mixture. The seeds mixtures sown are given on p. 542.

The original intention was to use the small plots, which were not laid out on the chessboard system, as demonstration and observation plots. Chiefly due to drought the large plots unfortunately failed, so that all information had to be derived from the small plots. In such small plots the experimental error is high for any yield comparison. Consequently

small differences are not stressed unless the botanical analysis and field notes support the superiority of certain plots. The chief aim was to follow the changes in the composition of the herbage and to note the competition between the various plants composing the pasture. No manures were added either before or during the experiment, so that any weakness would be readily shown. Much valuable information was obtained regarding the changes which occurred before the pasture settled. This is not only of importance to south-western areas, but makes a very interesting comparison with the results obtained by the Welsh Plant Breeding Station, which were published in 1928.

PLOTS: SEEDS SOWN AND CONDUCT OF EXPERIMENT.

The pasture plots were laid down in small areas 2 yards by 2 yards under similar soil and other conditions to the Hay plots (5). The Hay and Pasture plots were in close proximity. The beds were prepared and the seeds sown by hand in April 1923. No cover crop was used.

The small plots made a good start, with the exception of four, which lagged for two weeks before the seeds germinated. Soon after this dry spell, however, they all grew and continued to do so till the end of the experiment. It was this dry spell which upset the large plots, and unfortunately they never sufficiently recovered to make any work on them reliable.

The method adopted was to cut the plots and weigh green, being the same method adopted for the Hay plots (5). After weighing a sample was carefully drawn from each plot, dried indoors and analysed, the various ingredients being kept in separate packets; and when all analyses were finished the whole of the various ingredients were weighed on the same day so as to avoid any undue error. The margine effect is not great in closely-cut herbage. Even in 1927 there was no invasion from the turf permitted to grow towards the end of the experiment. The yields are left as pounds and ounces (lb./oz.) and not stated as weights per acre. Slight differences in weight are not stressed, as the plots were regarded more from the botanical than the agricultural point of view. Many of the deductions are based quite as much on field notes of the growing plants as on the actual weight data. Generally the two were very much in agreement, and it seemed doubtful if there would have been any marked difference in the results had the plots been repeated on the chessboard system. In fact the most valuable result of the investigation was the intensive study of what normally happens under field conditions in a considerable area of South Devon. It was most unfortunate that pressure

of other work made it impossible to attempt more than one analysis per annum, and that anything in the nature of a chemical analysis was out of the question.

There were two mishaps in the analysis, which prevent a complete series. In 1923 the samples drawn for analysis from 16*b*, 17*a* and 17*b* were, owing to a misunderstanding, thrown out along with some old Hay samples. Again, in 1924, the samples of 5*a* and 5*b* were partially burned by a student working in the laboratory. In both cases it was too late to obtain another sample, and botanical analyses of these plots are not available.

The frequent absence of the writer on advisory work rendered the harvesting and analysis rather difficult and, at times, impossible. It was therefore arranged that Mr C. A. Cosway, B.Sc., would conduct all the harvesting, weighing and analysis, and thus keep the sequence intact while the plots were in existence. To Mr Cosway I am deeply indebted; but for his assistance the work could not have been undertaken.

Changes in vegetation.

1923. (Tables I and III.) Although the plots were sown at the same time on soil carefully prepared and similar in every respect, yet four of the plots did not make a good start. Plots 4*a*, 5*a*, 6*a* and 7*b* were slightly later in covering the ground with vegetation. The seeds germinated slower, but the bad start retarded the progress of these plots for a time. This was particularly the case with 4*a* and, to a lesser extent, with 5*a*; the other two recovered rapidly and gave satisfactory yields. Plot 4*a*, having no Wild White Clover, naturally suffered more, owing to the loss of soil moisture; even 4*b* with Dutch White did much better.

The outstanding feature during this year was the extraordinary growth of 1*b*. The chief reason was the Italian Rye Grass giving it a good start. In 17*a* the Rye was on the heavy side, and depressed the other grasses and Red Clover. It brings out the importance of the proportions of the various grasses and clovers sown. Between most of the plots the difference in yield was not large and should not be over stressed, owing to the fact that the experimental error is considerable (Table I).

The composition of the yield at the time of analysis is interesting since, in 1*b*, Red Clover gave the chief weight with 34 per cent. of the yield, Italian Rye Grass coming next with 24 per cent. Plot 6*a* had 68 per cent. Perennial Rye Grass and only 11 per cent. Red Clover. Evidently 16 lb. Perennial Rye was too much for 4 lb. Red Clover. In

16 *b* Red Clover was the chief ingredient, Cocksfoot holding Rye in check, but in 17 *a* Italian Rye Grass was more prevalent than Red Clover. Had this not been the case 17 *a* would have given a much heavier yield. In all plots the yield was provided by Rye Grass and Red Clover in varying proportions.

1924. (Tables I and IV.) The yield this year was naturally higher than in 1923, and there were 4 cuts in place of 1 cut. The order of the plots was altered, 17 *a* heading the list, with 2 *a* close behind. In 17 *a* the Italian Rye Grass gave the early yield, but latterly Red Clover was the dominant plant. The analysis of the May cut is, of course, no true guide to the year's growth, but does indicate the growth at that stage. There was not a marked difference between most of the plots, but 7 *b* and 2 *b* fell behind the general yield of the plots. They subsequently occupied a much higher position, and gave reasonable yields.

Rye Grasses again gave much towards the yield, but Red Clovers gave much more in comparison. Black Medick or Trefoil also gave a high yield in some cases. Its semi-prostrate habit of growth rather depressed the yield of 16 *b* and, to a certain extent, also in 7 *a*. The high proportion of Black Medick in 16 *a* is very noticeable. The antagonism between Italian Rye Grass and Broad Red had given it a good chance. Its presence depends largely on competition. If competition between it and other plants is too fierce it makes no headway. This is practically realised by farmers, who generally include it as a stop-gap. A glance along the figures in Table IV (1924) shows that the rate of sowing is no guide to its yield. Timothy shows the same tendency, for its proportion varies from a trace to about 10 per cent. Plot 6 *b* was very interesting, since there was keen competition between the plants. Burnet seemed to make great progress and, unfortunately, rather retarded some of the other plants. Cocksfoot and even Timothy were evident in the yields and beginning to make their presence felt.

1925. (Tables I and V.) The yield this year was not so good as in 1924 (Table I). This is not due to any marked seasonal difference, but was evidently caused by the more frequent cutting, particularly during a period of drought when recovery is difficult (14). This year five cuts were taken instead of four the previous year. The frequent cuts seemed to afford the plants too little time to recover, and the short herbage suffered somewhat during the heat of the summer (20). The removal of cut herbage with no manures to make good the loss adversely affected both growth and recovery. After September there was nothing to cut. Normally, in the south-west, there is a considerable amount of growth after this date.

There was still considerable fluctuation in the yields of the various plots, but, apart from 1 *b* and 6 *b* which gave the heaviest yields and 5 *a* which was very light, the rest did not differ very markedly, taking all the factors into account.

The analysis made this year in March is only a guide to the nature of the early growth and not to the year's growth. Naturally Rye Grasses bulked large, especially Italian Rye. This is largely due to the constant cutting preventing flowering and seeding (13). The amount of Italian Rye Grass in the other cuts was not nearly so high. Even at this early stage Cocksfoot is steadily increasing, and it is clearly seen that there is marked antagonism between this grass and the Italian Rye (19). Wherever Italian Rye is dominant Cocksfoot is small in proportion, when Perennial Rye is dominant Cocksfoot gives a high yield (5 *a*, 6 *a*, 16 *b*, 17 *b*). In 7 *b* Cocksfoot is the dominant plant with 45 per cent., while Italian Rye is 10 per cent. In 17 *a*, where Italian Rye is 89 per cent., Cocksfoot is only 3 per cent. This year, although Rye Grasses gave considerably towards the yield, Cocksfoot gave much towards the end of the year, while Timothy was also present. Fescues and Chicory added a little in the later cuts, and there were now traces of Bent Grass in several plots. This year was a critical period; Rye Grass and Red Clover were not so plentiful, and Cocksfoot and Wild White had not quite developed sufficiently to occupy any bare space. It is just at this stage that Bent makes an appearance.

1926. (Tables I and VI.) This year it was decided to allow the plots to reach a good growth before cutting. This was to enable them to recover from the previous severe cuttings, and also to get an analysis which would provide a better guide to the actual growth and yield of the plots. Plot 6 *b* takes first place with 6 *a* following, and 17 *b* not far behind. The yield of the plot giving the heaviest weight was due to Burnet, Red Clover and Rye Grass, while in the others Cocksfoot and Red Clover were the chief factors giving weight, although Perennial Rye still gave a good addition. It will be noted that Burnet has seriously depressed the yield of Cocksfoot in 6 *b*. It is evident that Cocksfoot in most cases is the dominant plant, although Perennial Rye and Red Clover are still present in appreciable amounts. Another point in connection with early cutting is that Red Clover lasts much longer and gives good yields. Timothy is also present to some appreciable proportion, but the frequent cuttings and dry spells after cutting seemed to do it considerable harm. Italian Rye Grass is still present, but its amount is dwindling. The high proportion of Fescues in 5 *b* is very interesting; it shows that with the decrease

in quantity of Italian and Perennial Rye these grasses are able to add to the yield. It is evident that neither Cocksfoot, Timothy, nor Red Clover is responsible for the failure of this grass in most mixtures, but that the blame must be placed on the Rye Grasses (19). Italian Rye Grass has virtually disappeared from 17 *a* in spite of the heavy seeding.

The question of Buttercups and Burnet is a difficult one, for Buttercups seriously affected 1 *b*, while Burnet greatly reduced the yield of 6 *b*. In both cases these plants are not favourites with stock, and for that reason a deduction should be made from the total weight of the yield to give some indication of the yield available for stock feeding. This would materially alter the order of the plots as far as yield is concerned (Table I).

1927. (Tables I and VII.) The first cut was taken in May so as to enable the plots to make good growth, and to grow sufficiently before the summer drought set in. The season, however, being moist there was not the same danger, while the total yield was higher (Table I). No cut was taken after August, and the plots were turned in shortly after the early part of 1928. Perhaps one of the outstanding characteristics is that the yield of the two highest yielding plots is, in 1 *b*, composed of a high percentage of Buttercups ($22\frac{1}{2}$), and in 6 *b* to 45 per cent. Burnet. This greatly lowers the quality of the yield as previously pointed out, and naturally places these two plots much lower on the list than they are according to bulk yield (Table I). Burnet had a most depressing effect on many of the ingredients in 6 *b*, and would have been better omitted.

It is evident by this year that the plots are now tending to fall into groups (Table I). Not only so, but the more typical Cockle Park plots are giving the best results. Plot 3 *a* had not quite recovered from the early year's yield, but had materially improved, 4 *a* is suffering owing to lack of Wild White Clover to conserve the surface moisture and add nitrogen to the soil. Even the presence of Red Clover is flattering, since it gave a high proportion of a poor yield, while the 9 per cent. Dock and 6 per cent. Bent indicate the poor state of affairs in this plot (Table VII). Bent is on the increase, largely due to the decrease in Red Clover and Rye Grasses. The Perennial Rye Grass-Wild White Clover association is not yet quite stabilised and, therefore, not affording the necessary competition to keep Bent and other inferior grasses in check. Wild White Clover, however, would ultimately arrest this spread and improve the plots. The increase in Red Clover this year was noticeable; some of the seeds had recently germinated.

Cocksfoot is now definitely dominant in most plots. Rye has reached a more or less steady figure. Red Clover, though still persisting, is not

prominent and is steadily dying out, while Timothy is in the same condition, and as moisture was plentiful competition is the chief cause of failure. Wild White Clover is making great progress and indirectly adding to the yield. Even directly it has given in some cases an appreciable yield. This spread of Wild White Clover is bringing the various plots more into line, and in the course of time there would be very little distinction between many of the plots.

Yield considerations.

Although any strict comparison of yield with single small plots is quite out of the question, a brief consideration of the position of the plots is of some value. As the history and change in the vegetation were under observation, it is possible to draw some broad comparisons between certain of the plots, particularly with relation to the seeds mixture sown. The practical test of the success or otherwise of a pasture is the keep it affords, the amount of stock it can feed, and the gain in weight and condition of the stock, or the products of the stock (milk, wool or meat). In the present case one must rely on the yield and the composition of that yield.

Hay. (Tables I and II.) Hay yield has been estimated by adding the cuts for 1923 and April, May and June 1924. This would approximately be what a farmer might expect for the first cut, and most farmers take a cut of hay before pasturing the field.

Plot 1 *b* was by far the heaviest, with 17 *a* next. These were the plots with the heaviest seeding of Italian Rye Grass, 6 and 8 lb. respectively. The remainder of the plots, with the exception of the last three, 4 *a*, 5 *a* and 2 *b*, were arranged in three groups with little difference between them. Plots 2 *a*, 17 *b*, 3 *a* and 6 *b* formed the first group. Plots 7 *a*, 1 *a*, 16 *b*, 3 *b*, 6 *a* and 4 *b* formed the second group; and 5 *b* and 7 *b* the third group.

Plot 2 *a* occupies a rather high place, but with only 4 lb. of Cocksfoot the Rye Grasses had no serious competition except from the Clover. The same is true of 17 *a*, where there was 8 lb. of Italian Rye against the usual 10 of Cocksfoot, and 1 *b* when 6 lb. of Italian Rye Grass successfully competed with the 8 lb. of Cocksfoot and 4 lb. of both Red Clovers. Plot 3 *a* also occupies a high position, and here the Rye Grasses got well ahead of the Late Red Clover. It was evident, however, that its subsequent yields suffered as a result of this, as its position in the total yield was low. It will be noted that 16 *b*, the Cocksfoot mixture, with Broad Red did not give a very heavy yield, and 3 *b* with 6 lb. of both Clovers

was even lighter. Dry spells seem to affect Broad Red more than Late Red, while an injudicious mixture of both Reds reduces the yield. Both these points have been noted in county plots.

Considering the plots with no Italian Rye Grass 17*b* was the best, but 12 Perennial Rye, 8 Cocksfoot, and 2 Timothy gave the Late Red Clover a good chance, and it came away strongly in the later part of the year. Plot 6*b* (Elliot Mixture) owes its high position to Burnet and Chicory (19). Plot 16*b* was not too successful, owing to the lack of Late Red. Plot 6*a* did not occupy a high place, while 7*b* and 5*a* were very low. It seems, therefore, that unless there is some compensating factor or factors, the omission of some Italian Rye Grass tends to lower the yield. This is due to the absence of the shelter afforded by Italian Rye Grass during the early stages of the slower growing plants.

Pasture. (Tables I and II.) The pasture yield was assessed by adding all the cuts obtained after the Hay yield. This represents what would be available for grazing purposes.

As far as pasture yield is concerned, 1*b* and 6*b* are easily first, but the high proportion of Buttercups in 1*b* and Burnet in 6*b* lowers the value considerably. Stock are not too partial to Burnet, as was recorded by Elliot (4) and Carruthers (3), although there seems some doubt on the matter. Personally we find it seldom eaten if better food is available. Plots 17*a* and 6*a* are next, both plots with typical Cockle Park Mixtures, 17*a* having 8 lb. of Italian Rye while 6*a* had none. Plot 2*b* ranks next, and the rest follow in fairly even sequence, till 4*a*, which lags far behind. Plot 4*a* had no Wild White Clover, and that is the explanation of its failure. Plot 4*b*, just above it, had also no Wild White but 1 lb. Dutch White.

Examining next those plots with no Italian Rye Grass it will be noted that, with the exception of 6*b* and 6*a*, they do not occupy a high position. Plot 6*b* was compensated by Burnet and Chicory, while 6*a* had more Perennial Rye (16 lb.). Plot 17*b* had a light sowing of Perennial Rye (12 lb.) and 7*b* was still on the light side (14 lb.), although it had 2 lb. of Wild White Clover. This last plot, however, was rapidly improving, and would later have taken a higher position. Plot 16*b* was upset by the use of Broad Red Clover, while 5*a* had a light sowing of Perennial Rye (14 lb.) and Cocksfoot (8 lb.), instead of the more usual 16 lb. and 10 lb. respectively.

It is evident, therefore, that although some Italian Rye Grass does much to help the start of the pasture, provided the mixture is well balanced, any primary disadvantage will tend ultimately to diminish, other things being equal.

Total yield. (Tables I and II.) Considering the total yield, 1 *b*, 6 *b*, 17 *a* and 6 *a* stand well above the others. After this the plots fall into two large groups, 7 *a*, 17 *b*, 2 *a* and 7 *b*, with very little between them. Then after these there is the group of plots 1 *a*, 2 *b*, 3 *b*, 5 *b*, 3 *a* and 16 *b*. With a considerably smaller yield come 4 *b* and 5 *a*, then far behind other plots, 4 *a*.

Plots 1 *b* and 6 *b* occupy a rather flattering position, as already indicated. This means that 17 *a* and 6 *a* (Cockle Park) would be the best plots. If we consider the other Cockle Park plots, 7 *b*, which is in the first large group, had no Italian Rye Grass but 2 lb. of Wild White Clover. Plot 17 *b* had only 12 lb. Perennial, 8 lb. of Cocksfoot, $\frac{3}{4}$ Wild White Clover and was placed in the first large group. Plot 16 *b* had 4 lb. of Broad Red Clover and no Italian Rye Grass. Broad Red rather upset the balance in 1923, and the plot suffered on this account; and it was only latterly that it began to take a better place. It occupies the lowest place in the second group of plots. Plot 3 *a* had 8 lb. of Cocksfoot instead of the usual 10, and is placed in the second group just above 16 *b*. Plot 7 *a* is rather interesting, since only 2 lb. of Late Red Clover were sown. Rye Grasses dominated the plot till the later phases, when Cocksfoot increased in a remarkable manner. Red Clover gave a steady but never a high yield. Cocksfoot developed late, due to the predominance of Rye Grasses and insufficient Red Clover to keep the Rye in check. Plot 7 *a* took the highest position in the first group of plots. Thus it seems that the typical Cockle Park mixture of 16 Rye Grasses, 10 Cocksfoot, 4 Timothy, 4 Late Red, 1 Wild White, etc., is the most satisfactory and, other things being equal, gives the better yield (17). The high position of 2 *a* is interesting, since it had only 4 lb. of Cocksfoot. It will be seen that in 1924 and 1925 Red Clover bulked largely in the cuts. Rye and Red Clover held Cocksfoot in check till very late in the year 1926. It is rather significant that 4 *a*, by far the poorest plot, had no Wild White Clover or White Clover. Plot 4 *b*, with 1 lb. of White Clover, was also poor. Plot 5 *a* was slightly behind 4 *b*, but had only 14 instead of 16 lb. of Rye Grass and no Italian Rye, while there were 8 lb. of Cocksfoot instead of 10, and no extra seeding of other things to make up the deficiency.

Plot 3 *b* with $6\frac{1}{2}$ lb. of Red Clover (4 Broad Red and $2\frac{1}{2}$ Late Flowering) does not occupy a high position. Increased seeding may actually lead to a decrease. The mixing of Broad Red and Late Flowering Red Clovers is not often a successful procedure in the south-west. This had previously been noted in certain county plots (19).

It is quite evident from the figures (Table II) that the well-balanced seeds mixture gives the best result, and that the Cockle Park type is

superior, although $2a$ takes a high place considering the fact that only 4 lb. of Cocksfoot were sown. The high yields of $1b$ and $6b$ are misleading, since a large proportion of the bulk is unpalatable material. The figures show how important is the competition of the ingredients of the seeds mixture. Any plants in undue proportion or of too great competitive power (*e.g.* Burnet) may not only upset the vegetation, but seriously reduce the yield of palatable plants. The importance of Rye Grasses and Red Clover in the early stage and Cocksfoot and Wild White Clover in the later stage is very evident.

Discussion. (Tables III–VII.)

The failure of the large plots and the success of the botanical plots provided a very valuable object lesson. The field where the large plots were laid down was about the same as regards soil conditions as the botanical plots, although the latter were perhaps slightly better. The large plots had the advantage of a cover crop, less exposure, and adequate manuring. The tilth of the botanical plots was finer and the soil was cleaner. The botanical plots were on a good slope exposed to the sun. Hence, apart from the disadvantage of no cover crop, no manures, and more exposure, the botanical plots had only better tilth and some greater freedom from weeds. But it was this which just tilted the balance in the direction of success for the botanical plots. It brings home very clearly how small may be the margin between success and failure in field work under practical conditions, where there are so many and often conflicting factors at work. It is on this account that plot work is so valuable in often indicating the direction in which success may lie.

In the early stages it was noticeable that the plots with Italian Rye Grass not only gave the most vegetation, but that the early growth of Italian Rye sheltered the other plants (19), did much to conserve soil moisture, and improved the germination of the slower growing grasses and clovers (19). The conservation of moisture may perhaps be a little on the high side for, by 1927, plot $1b$ was covered with *Ranunculus repens*. In spite of this there was an appreciable amount of Wild White Clover. During drought spells the initial advantage of the Italian Rye Grass plots was maintained, since the moisture conserved in the early stages enabled plants to make more growth and to root deeper. This is a most important point in the use of a small quantity of Italian Rye Grass, which has not received the attention it deserves in those areas where summer drought is likely to occur (5). In areas where summer drought occurs or where the soil is dry, Italian Rye Grass is sometimes included,

because it is said to give an early bite and that it gives more keep. The real truth is that, apart from an early bite, the initial start and protection afforded by the presence of Italian Rye Grass is reflected in the greater and fresher growth of the other ingredients. In short, that the improvement is not so much directly as indirectly due to Italian Rye Grass. Moreover, this effect lasts for a considerable time and not only during the first season. Many more seedlings survive under the protection afforded and consequently a much closer turf is obtained. This also means that if the grazing (or cutting) is sufficient, Wild White Clover will flourish and cause a still further improvement. Wild White Clover grows extremely well in the early stages under the shelter of some Italian Rye Grass. Italian Rye Grass is the first stage, and Wild White Clover, the subsequent stage, is the solution of the drought difficulty as far as pasture production is concerned (16).

It is very noticeable that the poorest plots were those with no Wild White Clover (12) or no Italian Rye Grass. It is a well-known fact that Wild White Clover must have a reasonable supply of moisture, and that unless this is available in the early stages many young plants die. Once Wild White Clover is established, it can withstand considerable drought as the leaves shade the ground and prevent undue evaporation. This also makes for better growth of the grasses, and explains why the best plots were those with Italian Rye Grass, Wild White Clover (also the plot with Burnet and Chicory) plus Cocksfoot (4). These were the plots which showed the greatest resistance to drought. The staying power of Italian Rye Grass when not allowed to flower or seed is remarkable, and is by no means the only instance we have noted in the south-west of England (5).

Timothy showed the same failure to make good and last which is characteristic of many South Devon pastures. There are two factors at work; the first being competition and the second moisture. Both these factors operate largely in the early years. Although from a germination point of view it never makes such progress as most of the other grasses, its staying ability is not good under grazing conditions. In a wet summer it does much better. The fact that, in the same soil a few yards away, 10 lb. of Timothy and 10 lb. of Late Flowering Red Clover per acre both grew and lasted well under Hay conditions (5, 19) raises the question as to whether a heavier seeding might help (19)? The frequent cutting did much to weaken the plants and may partially explain why even after a good start this grass is usually short-lived in pastures. The grazing or frequent cutting, especially during dry spells, when Timothy cannot recover so quickly, is probably the explanation of its absence in many pastures

where it was originally sown. Another point is that sheep in particular are very apt to over-graze Timothy in a pasture, and thus exhaust it beyond recovery.

It was very evident from the beginning that cutting was not a satisfactory substitute for grazing (7), even although the cutting was done as close as a grazing animal (10). Even plucking with the fingers made little difference in the end when tried on some other plots. The soil very soon became uneven and worm castings were frequent. This meant the exposure of a greater surface of ground, which was very unfortunate after a cut in the dry weather. More moisture was lost than there should have been, while the vegetation tended to become slightly tufted in spite of the frequent cuts. It also affected Wild White Clover, since it reduced the moisture available. Rolling is essential and plays a very important part in all grassland management (15). Even if there is no rolling, the constant treading of the stock greatly assists the vegetation. It is unnecessary here to go into the physical effects on the soil of rolling, and treading by stock, and also the effects on the vegetation. It is a point which will have to be considered in all plot work of a pasture nature, and rolling of some sort will have to be introduced to eliminate this source of error.

On certain soils rolling, if excessive, may do harm and is sometimes blamed for encouraging Bent. This may be so, but much depends exactly when the rolling is done and under what conditions. In most cases a dressing of lime would prevent any bad effects, provided the rolling is done judiciously. The fact that the field from which the plots were derived had very little Bent shows that, in the present case, rolling would not have produced that result (8). This is still further emphasised in that the surrounding paths which were allowed to grow a turf during the last year of the plots (1927) showed hardly a trace of Bent, but were almost a closed association of Perennial Rye Grass and Wild White Clover (2, 19).

Another important point is that those plots without Wild White Clover naturally suffered from the continuous removal of material, with neither manures nor the droppings of stock being added to the soil. The removal of nitrogen was very heavy, and only those plots which had Wild White Clover had any nitrogen returned, for Red Clovers had died out or fallen to such a low figure that little or nothing was being added to the soil from that source. This points to the question of inadequate competition with Bent, owing to reduced fertility of the soil.

A rather noticeable point was that the pasture plots contain few

weeds in comparison with the Hay plots (5). In the early stages the pasture plots had a large number of weed species. This was to be expected as the vegetation was short, frequently cut, and until a turf had developed dormant weed seeds got every chance to assert themselves (2). In the Hay plots the tall growth shaded out most of the smaller weeds, and it was only in the periods following the cuts that most weeds got a chance. The Hay plots had a smaller number of species but a larger bulk of weeds (5). Apart, however, from larger weeds like Thistles, Docks and Sow Thistles, which would bulk out of all proportion in a small plot, the most important weeds were grasses such as Bent, Soft Brome, and Yorkshire Fog, but these made little progress in the pasture plots. Buttercups were too high in 1*b* and one other plot, but otherwise this plant did not cause any trouble. The case of Bent is an important one, for it is one of the chief problems in many Devon grasslands. Lack of manures or the droppings of stock certainly help Bent in the pasture plots. Where frequent cutting encourages bottom growth competition is much greater. Another important factor is that, with a short herbage, Wild White Clover usually shows to great advantage (1, 3, 10, 11). Wild White Clover and Bent always compete very fiercely (6). If Perennial Rye Grass is associated with Wild White Clover (and, in some cases, Rough Stalked Meadow Grass) on fairly good soil, Bent will never make serious headway, provided the pasture is properly grazed and treated well (19). Perennial Rye Grass alone will seldom hold its own against Bent, unless aided by a Clover (6). The Bent problem is really a question of competition between plants. It is rather curious that the two plots with most Bent in 1927 were those with half the usual sowing of Cocksfoot and Timothy (2*a* and 2*b*). In Hay, so long as there is a good top canopy, Bent will be kept under, but if the top canopy is removed for any space of time and Bent once gets a start, only very strenuous efforts will keep it in check. Its late "first growth" often coincides with the period just following the Hay cut, while the late growth of this plant in autumn gives it a very good chance. Once it gets up it can, as it were, superimpose tuft on tuft of growth till practically everything else is smothered. The spread of Bent (Creeping Bent) is not so much due to seeding as to vegetative parts remaining in the soil, even after the land is broken up (2*a*) Only many years of arable cropping would destroy this mode of propagation and spread. Surface moisture also greatly aids this grass especially in autumn. That is why "laying up for winter keep" has such unfortunate results. It will be seen that in 1927 Bent reached 10 per cent. in only two plots, and that was aggravated by a year of plentiful moisture. The

steady increase in Wild White Clover would have more than held it in check, had the plots remained down for a longer period. Where not-dominant Bent is a plant which fluctuates very much in amount from year to year and is considerably influenced by the season, not directly so much as indirectly as the season affects its competition.

Soft Brome failed to make any progress. The close and frequent cutting prevented this grass from making any growth, and never allowed it to set seed (19). The presence of this grass in pastures is usually an indication of neglect or insufficient grazing. In hay and meadow lands its success depends on its ability to produce and set seed before the hay is cut. Yorkshire Fog, although present, never made itself very evident. Here again constant cutting and the failure to seed prevented this plant from becoming plentiful (19). In the Hay plots its ability to seed before the cutting period was its chief asset for success. In both cases a powerful factor against the success of those plants in the pasture plots was that the competition of other grasses and clovers was too severe.

It is pretty evident that Italian Rye Grass is the grass which has the most depressing influence on most of the other plants, although it has a most beneficial effect in the early stages of growth (19). Tall Oat is quite as aggressive if not more so, as it generally lives longer. Next in order ranks Perennial Rye Grass, then follows Cocksfoot (19), and Bent must be reckoned the next aggressive. In fact Bent may be placed much higher, since once it gets a chance it can compete with most grasses and clovers, especially if the conditions are unfavourable. Rough Stalked Meadow Grass is slightly aggressive in wet seasons (19). The dry spells are often fatal to the success of this grass, and it naturally raises the question of substituting this grass entirely or partly by Smooth Stalked Meadow Grass where summer drought is probable.

Red Clover competes fiercely with both Rye Grasses. Cocksfoot and Red Clover compete, but this is not so marked, since towards the end of the second year and the beginning of the third Cocksfoot is attaining its maximum growth, while Red Clover is usually dying out (Tables V and VI) (19). In the early stages of its growth Cocksfoot is retarded by Red Clover, but in the south-west this is more than compensated for by the shelter and moisture conservation given by the Clover growth (19).

Meadow Fescue and even Tall Fescue are very sensitive to competition with Rye Grasses, and also to a less extent with Clovers (19). Competition between Fescue and Cocksfoot does not seem to be very marked since Fescue did well even after Cocksfoot was the dominant plant (Tables VI and VII) (19). The late appearance of Meadow and Tall Fescues

is rather puzzling. It must be either delayed germination or arrested growth and development. In the plot concerned (5*b*) it was a case of delayed germination. In their young stages Fescues can be easily overlooked and confused with Rye. In the south-west the late appearance seems to be chiefly a case of delayed germination (19). This was frequently noted in fields which had been down to grass for several years. It was only some years after that Fescue made an appearance. It is, however, a matter requiring more attention. Several instances of delayed germination in Late Flowering Red Clover and Rough Stalked Meadow Grass have been observed in the south-west area. The germination of Red Clover and Rough Stalked Meadow Grass seeds is considerably affected by lack of moisture or a low temperature. The result is that one to three years later these plants come up and seriously affect some crop (19). In one instance an Oat crop was practically smothered by the germination of Red Clover seed, sown three years previously.

Black Medick, like Rough Stalked Meadow Grass, is a plant much influenced by season and competition. Both these plants are indigenous to the soil of many districts in South Devon, and in moist growing seasons may greatly interfere with crops.

A very noticeable feature of the plots is the failure of Alsike Clover. The chief cause of this is the fierce competition with Red Clover (19). Farmers include Alsike Clover for "filling up gaps if the Red Clover fails," but it is evident that under conditions similar to the pasture plots it is a waste of seed and money to include it. Even the Hay plots (5) demonstrated this fact. There are some districts in the south-west where owing to greater moisture Alsike Clover does so well that it competes with Red Clover and reduces the yield of Hay.

The succession of vegetation in the pasture plots affords quite an ecological study. The stages may be divided into three. First there is the opening stage when Rye Grasses and Red Clovers dominate the vegetation. This was the case from 1923 till the end of 1924, and the early part of 1925. The second stage commenced in 1925 when Red Clovers and Rye Grasses were decreasing and Cocksfoot, Timothy (if present), Wild White Clover and Fescues show an increasing amount. This phase continued for about another year (till 1926). It is a very critical period in pasture formation, for unless the increase of the slower maturing species equals the decrease in the quick and shorter-lived grasses, interlopers fill up the gaps and may seriously affect the pasture. The use of plants like Black Medick for gap-filling is very evident. Sometimes such gap-fillers may do too well and fill more than the gap and depress the yield. It is at this

stage that Bent, inferior grasses, and weeds make their presence felt. The two cuts instead of several in 1926 rather favoured the inferior grasses and weeds, but fortunately they were successfully held in check by the useful plants. The last stage began about the end of 1926 and the early part of 1927. Perennial Rye Grass and Red Clover had decreased but Cocksfoot held a dominating position above (19) and Wild White Clover below. Perennial Rye had reached a steady proportion and would probably have increased if left. Wild White Clover covered about 50 per cent. of the ground space in most plots and Bent was successfully held in check (19). The critical stage was past, and it was quite evident that the plots were settling down to a more permanent flora closely resembling the original field. Fescues gave a good quota in 5*b* and the moisture of 1927 enabled Yellow Suckling Clover to make an appearance. Bent reached a fair proportion in three plots and weeds were present in some of the plots. The best plots of the typical Cockle Park Mixture showed a strong resemblance to each other, and differed from the original pasture only in the larger proportion of Cocksfoot present.

While in the pasture plots Cocksfoot and Timothy were not in great evidence till the close of the year 1924 and afterwards, yet when sown alone or with one other ingredient they grew well in the first and second year (19). Now Cocksfoot germinates well and grows well so it cannot in this case be delayed germination. Timothy, though not so robust and also much affected by the lack of moisture, also grows well with Red Clover. In actual speed of germination in the field, Rye Grasses are faster than Cocksfoot, and Cocksfoot faster than the Timothy. It is evident though that Rye Grasses overshadow the slower growing Cocksfoot and still more severely shadow Timothy (19). Timothy seedlings were not plentiful in the seedling stage of the plots, and it rather suggests that, when moisture is not plentiful, the field germination may be very low. In the Hay plot with Red Clover (5), Timothy—possibly with the shelter and no grass competition—did much better and the field germination was good (19). This may, perhaps, in some instances, be the explanation of the late appearance of Fescue under competition with Rye grasses.

Excluding Bent, Yorkshire Fog and Soft Brome Grasses the other weeds present in the pasture plots were: *Cerastium triviale*, *Stellaria media*, *Sinapis arvensis*, *Taraxacum officinale*, *Sonchus oleraceus*, *Crepis taraxacifolia*, *Hieracium* spp., *Senecio vulgaris*, *Bellis perennis*, *Sagina procumbens*, *Anagallis arvensis*, *Veronica* spp., *Sherardia arvensis*, *Linum catharticum*, *Geranium dissectum* et *molle*, *Convolvulus arvensis*, *Poa annua*, *Musci* spp.

The following weeds were in the old pasture: *Cnicus arvensis*, *Rumex obtusifolius* et spp. (near gate), *Geranium molle* et *dissectum*, *Taraxacum officinale*, *Convolvulus arvensis*, *Plantago major* (near gate), *Poa annua* (near gate).

Those weeds in the pasture plots and not in the original pasture had been introduced with Hay, implements and stock. The only one of outstanding interest is *Crepis taraxacifolia*, which probably spread by wind since the nearest plants were growing about 200–300 yards away. *Linum catharticum* occurred in a neighbouring pasture, and the seeds had doubtless been carried by soil. The constant moving of people round the plots must have introduced many seeds, both grasses and weeds.

It was very noticeable by 1927 that the best of the pasture plots were rapidly approaching in appearance the original pasture lying outside the area of the plots. The two chief differences were (1) the smaller quantity of Wild White Clover which, however, was steadily increasing, and (2) the greater quantity of Cocksfoot in the plots (18). As a result, Perennial Rye was not so plentiful in the plots. Had the plots remained down for another two years and the growth kept short, it would by the close of 1929 (judging by the trend of progress) have been exceedingly difficult to distinguish between the bulk of the old permanent pasture and the recent plots. Had these plots been manured the improvement would have been still more marked.

It brings out very clearly the importance of management in the success of grassland (19). Even those plots which were behind the others would in time, by careful treatment, have been improved, till finally they approached if not equalled the old pasture. On poorer land this could not have been done, for there Bent would inevitably have gained too great a footing, and the history of the pasture would be still another of those cases of "decline and fall."

The plots generally were satisfactory, and Bent had not made any great progress in spite of constant cutting and no manuring. It shows that it takes prolonged bad treatment to so exhaust a pasture that Bent, Yorkshire Fog and Weeds give the bulk of the vegetation. The condition of many of the poorer pastures in the south-west of England (6) points to a long period of grazing (7) and perhaps Hay cutting (9), without any manuring, or no adequate manuring, to make good the loss of plant food in the soil. A point of considerable practical importance is the question of the Hay cut during the early development of a pasture. Farmers generally like a good Hay yield in the early stage of growth. This frequently leads to the selection of a mixture which will give a very heavy

Hay crop. Now the mixture which gives a heavy Hay crop is usually not the best mixture for a pasture which is to be left down for several or many years. As a result, for an initial advantage of a heavy Hay yield, the yield and condition of the pasture is often much reduced, if not at times seriously affected. This may be illustrated by reference to Table II. In Hay yield 2*a* and 17*b*, among others, took a relatively high position but were much lower in pasture yield. In pasture yield 2*b* and 7*b* gave a good yield in comparison with other plots, but occupied a low position in the Hay yields. In 2*a* and 17*b* grasses were reduced and Red Clover had less competition, while in 2*b* there was only 2 lb. of Timothy, while 7*b* had an extra quantity of Wild White Clover. Where much more drastic alterations in the mixture are made the result is obvious. Even cases of taking Hay crops for two years in succession is not a very rare occurrence. The practice can be justified neither botanically nor financially. The removal of a Hay crop means a heavy loss to the soil in plant food and also, owing to the shade cast by the crop, surface growth is depressed. The combination of these two is against good turf formation, and if aggravated by two Hay cuts makes the production of a pasture out of the question. The combination of exhaustion, or at least depletion of soil fertility, and the bare spaces are ideal conditions for weeds and inferior grasses to gain an entrance and compete far too successfully with the grasses and Clover sown. Plots 6*b*, 1*b* and 17*a* (Table II) indicate very clearly that a typical Cockle Park Mixture gives not only a good pasture (19) but a satisfactory initial Hay cut. If this mixture is too severely altered to give added weight to Hay, it succeeds at the expense of the pasture. In other words, the income over many years is seriously reduced for an immediate and very inadequate lump sum.

Summary.

The best seeds mixtures were of the Cockle Park type.

In the initial stages, good soil tilth and freedom from weeds may outweigh certain disadvantages in the formation of a pasture.

The shelter afforded by the quick germination and growth of Italian Rye Grass is very important in areas affected by summer drought. Soil moisture is conserved and slower developing seeds show an increased germination and growth.

In the later phases of turf formation, Wild White Clover takes the place of Italian Rye in conserving surface moisture.

The passing of Red Clover and decrease of Rye Grasses must be

compensated for by the growth and spread of Cocksfoot, Wild White Clover, Rough Stalked Meadow Grass and other grasses.

The plots showed three phases in vegetation before a more stable flora was developed.

The order of aggressiveness in the plots was: Italian Rye, Perennial Rye, Red Clover, next Cocksfoot followed by Wild White Clover, Bent and Rough Stalked Meadow Grass, then Timothy, Tall and Meadow Fescue.

Burnet is too aggressive; Chicory is not so aggressive, is more palatable and quite as resistant to drought.

Cocksfoot, Fescues and Wild White Clover are good drought resisters when once established.

Tall and Meadow Fescues are depressed chiefly by Rye Grasses. In the plots studied, the late appearance of Fescues was due to delayed germination.

Alsike Clover was a failure, and Timothy was not a success.

A high seeding of Red Clover, or mixtures of Broad Red and Late Flowering Red Clovers are not always successful.

Frequent or continuous cutting and removal of vegetation depresses growth in the following year.

Lack of rolling or treading in small plots is a source of experimental error.

The weed flora of the Pasture plots was small and in marked contrast to the Hay plots.

In spite of initial differences, under similar treatment there is soon a marked tendency to develop a flora of the same proportions in all plots.

Altering a seeds mixture suitable for a long ley or permanent pasture so as to obtain a heavy Hay cut from the early growth is unsound. It endangers turf formation, encourages inferior grasses and weeds, and depresses the useful plants.

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Seeds mixtures used in plots. Expressed in pounds per acre.

Species	1a	1b	2a	2b	3a	3b	4a	4b	5a	5b	6a	6b	7a	7b	16b	17a	17b
Lolium perenne	14	10	14	14	14	14	14	14	14	10	16	10	14	14	16	8	12
italicum	2	6	2	2	2	2	2	2	0	2	0	2	2	0	0	8	0
Dactylis glomerata	8	8	4	8	8	8	8	8	8	8	10	8	8	10	10	10	8
Phleum pratense	4	4	4	2	4	4	4	4	4	3	4	4	4	4	4	4	2
Festuca pratensis	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0
Poa trivialis	½	½	½	½	½	½	½	½	½	½	0	½	½	4	0	0	0
Festuca elatior	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0
Trifolium pratense	1½	1½	1½	1½	0	4	1½	1½	1½	1½	0	1½	0	0	4*	0	0
pratense[1]	2½	2½	2½	2½	4	2½	2½	2½	2½	2½	4	2½	2	4	0	4	4
repens[2]	1	1	1	1	1	1	0	0	1	1	1	1	1	2	1	1	¾
repens	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
hybridium	1	1	1	1	1	1	1	1	1	1	0	1	0	0	0	0	3
Medicago lupulina	1	1	1	1	1	1	1	1	1	1	1	2	3	0	1	1	1
Plantago lanceolata	0	0	0	0	0	0	0	0	0	0	0	1	3	0	0	0.	0
Cichorium intybus	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0
Poterium sanguisorba	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0
Petroselinum sativum	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Achillea millefolium	0	0	0	0	0	0	0	0	0	0	0	¼	0	0	0	0	0
Anthyllis vulneraria	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0

* Broad Red Clover. [1] Late Red Clover. [2] Wild White Clover.

Table I.

Details of plot yields for period 1923–7.

	1923	1924					1925						1926			1927			5 years
	Oct.	April	May	June	Aug.	Total for year	Mar.	May	June	July	Sept.	Total for year	June	Aug.	Total for year	May	Aug.	Total for year	Total for all cuts
1*a*	4 10	2 6	7 0	4 3	7 9	21 2	1 14	4 10	3 9	2 14	5 1	18 0	9 1	2 8	11 9	10 8	8 19	19 11	75 0
1*b*	16 0	3 6	6 8	3 14	7 5	21 1	2 15	4 12	4 0	5 6	6 14	23 15	12 1	6 5	18 6	19 0	12 0	13 0	110 6
2*a*	3 8	6 0	6 2	5 6	7 1	24 9	2 9	6 14	2 6	2 5	4 2	18 4	11 12	1 15	13 11	10 0	8 12	18 12	78 12
2*b*	3 0	3 3	5 4	2 3	4 9	15 3	2 5	4 13	3 0	2 15	4 0	17 1	11 12	2 15	14 11	14 8	10 0	24 8	74 7
3*a*	4 0	5 5	6 8	4 3	5 9	21 9	2 0	5 12	2 8	2 1	3 11	16 0	11 10	1 5	12 15	10 4	8 8	18 12	73 4
3*b*	3 1	4 0	6 14	3 11	6 1	20 10	1 13	4 9	2 1	2 4	3 3	13 14	11 9	2 6	13 15	13 8	10 2	23 10	75 2
4*a*	2 9	1 15	5 4	5 6	5 7	18 0	1 6	5 10	2 3	2 0	3 3	14 6	8 12	1 4	10 0	8 8	4 2	12 10	57 9
4*b*	2 6	2 10	6 8	5 14	4 11	19 11	1 12	4 9	1 13	2 10	3 1	13 13	11 8	1 12	13 4	10 0	9 0	19 0	68 4
5*a*	3 13	2 4	6 5	3 6	6 13	18 12	0 14	4 13	1 13	1 15	3 2	12 9	11 0	1 10	12 10	11 0	8 0	19 0	66 12
5*b*	3 2	2 10	6 10	4 1	4 15	18 4	2 12	5 13	1 12	2 7	3 5	16 1	12 15	2 1	15 0	11 0	10 2	21 2	73 9
6*a*	5 2	2 7	6 11	3 5	6 7	18 14	2 2	7 10	1 10	2 7	4 1	17 14	16 11	2 7	19 2	15 8	10 0	25 8	86 8
6*b*	6 2	2 10	6 5	4 8	6 13	20 4	2 12	6 10	1 14	3 15	4 7	19 10	17 10	4 4	21 14	16 8	15 12	32 4	100 2
7*a*	5 0	2 3	6 4	5 2	4 3	17 12	2 0	6 7	1 13	2 9	4 6	17 3	13 15	1 6	15 5	14 8	10 12	25 4	80 8
7*b*	4 0	1 10	6 8	4 2	4 5	16 9	2 0	6 15	1 9	2 3	3 6	16 1	13 13	1 11	15 8	14 8	11 8	26 0	78 2
16*b*	6 2	1 6	6 4	4 6	5 9	17 9	1 4	4 8	2 8	1 9	3 9	13 6	12 6	2 4	14 10	11 0	10 4	21 4	72 15
17*a*	5 11	4 8	7 1	6 5	7 1	24 15	3 4	4 12	3 0	2 6	4 6	17 12	13 9	2 15	16 8	16 12	11 8	28 4	93 2
17*b*	5 6	1 6	6 8	6 14	5 9	20 5	1 10	4 9	2 10	2 4	3 11	14 12	15 5	1 12	17 1	11 0	11 8	22 8	80 0
	Cut for analysis		Cut for analysis				Cut for analysis						Cut for analysis			Cut for analysis			

Table II.

Plots arranged according to yield: Hay, Pasture, and Total.

Hay yield			Pasture yield			Total yield			Pasture 1927		
	Weight			Weight			Weight			Weight	
Plot	lb.	oz.	Plot	lb.	oz.	Plot	lb.	oz.	Plot	lb.	oz.
1*b*	29	12	1*b*	80	10	1*b*	110	6	6*b*	32	4
17*a*	23	9	6*b*	80	9	6*b*	100	2	1*b*	31	0
2*a*	20	10	17*a*	69	9	17*a*	93	2	17*a*	28	4
17*b*	20	2	6*a*	68	15	6*a*	86	8	7*b*	26	0
3*a*	20	0	2*b*	64	13	7*a*	80	8	6*a*	25	8
6*b*	19	9	7*a*	61	15	17*b*	80	0	7*a*	25	4
7*a*	18	9	7*b*	61	14	2*a*	78	12	2*b*	24	0
1*a*	18	3	17*b*	59	14	7*b*	78	2	3*b*	23	10
16*b*	18	2	2*a*	58	2	1*a*	75	0	17*b*	22	8
3*b*	17	10	5*b*	57	2	2*b*	74	7	16*b*	21	4
6*a*	17	9	1*a*	56	13	3*b*	74	2	5*b*	21	2
4*b*	17	6	3*b*	56	8	5*b*	73	9	1*a*	19	11
5*b*	16	7	16*b*	54	13	3*a*	73	4	4*b*	19	0
7*b*	16	4	3*a*	53	4	16*b*	72	15	5*a*	19	0
4*a*	14	12	5*a*	52	0	4*b*	68	12	2*a*	18	12
5*a*	14	12	4*b*	51	0	5*a*	66	12	3*a*	18	12
2*b*	13	10	4*a*	42	13	4*a*	57	9	4*a*	12	10

Table III. 1923.

Analysis of chief ingredients of plots. Percentage by weight of plants present in October cut.

Species	1*a*	1*b*	2*a*	2*b*	3*a*	3*b*	4*a*	4*b*	5*a*	5*b*	6*a*	6*b*	7*a*	7*b*
Festuca {*elatior* / *pratensis*	—	—	—	—	—	—	—	—	—	2·9	—	—	—	—
Lolium perenne	50·0	11·6	44·9	45·6	52·3	46·5	41·1	35·3	44·9	28·5	67·8	40·6	45·3	60·3
italicum	17·0	24·4	19·9	17·7	17·2	16·1	8·5	12·1	3·6	20·7	—	22·0	9·5	3·0
Dactylis glomerata	2·0	T.	1·1	10·0	2·3	2·5	2·9	3·3	2·5	4·3	2·7	1·1	T.	9·1
Phleum pratense	—	2·0	—	—	—	—	—	3·2	—	T.	1·8	T.	T.	1·5
Poa spp.*	2·0	1·3	4·1	—	—	—	—	—	—	—	—	—	—	—
Agrostis palustris, Huds.	—	—	—	3·6	—	1·1	4·9	1·4	—	1·4	—	—	T	—
Trifolium pratense	14·4	34·5	13·9	8·2	7·6	16·5	19·3	22·3	22·5	19·9	11·0	7·5	2·2	20·7
repens	—	—	—	—	—	—	—	—	—	—	—	—	—	—
hybridium	—	—	3·2	1·2	T.	1·2	1·3	2·4	1·8	T.	—	T.	—	—
Medicago lupulina	12·5	23·3	6·9	9·6	13·7	11·7	18·3	14·8	19·1	18·3	12·5	9·5	16·3	T.
Poterium sanguisorba	—	—	—	—	—	—	—	—	—	—	—	9·7	—	—
Cichorium intybus	—	—	—	—	—	—	—	—	—	—	—	2·4	—	—
Ranunculus repens	—	—	—	—	3·7	1·4	1·0	—	—	—	—	—	—	—
Carduus arvensis	—	—	—	—	—	—	—	—	—	—	1·0	—	—	—
Rumex spp.	—	—	—	—	—	—	—	—	1·2	—	—	—	—	0·6
	—	—	—	—	—	—	4·9	—	—	—	—	—	3·0	—
Cerastium spp.	—	—	—	—	—	—	—	—	—	—	—	—	—	3·9
Onobrychis sativa	—	—	—	—	—	—	—	—	—	—	—	T.	—	—
Plantago lanceolata	—	—	—	—	—	—	—	—	—	—	—	—	23·7	—

No figures for Plots 16*b*, 17*a* and 17*b*.

* Chiefly *P. trivialis*.

Table IV. 1924.

Analysis of chief ingredients of plots. Percentage by weight of plants present in May cut.

Species	1*a*	1*b*	2*a*	2*b*	3*a*	3*b*	4*a*	4*b*	5*a*	5*b*	6*a*	6*b*	7*a*	7*b*	16*a*	17*a*	17*b*
Lolium perenne	34·6	8·6	41·50	51·1	62·7	34·9	64·0	46·1	—	—	48·4	28·6	36·90	62·03	30·0	12·5	69·09
italicum	T.	30·2	6·0	6·0	20·1	19·0	T.	T.	—	—	—	T.	10·80	—	—	50·4	—
Dactylis glomerata	4·80	6·20	3·10	4·9	1·1	1·1	2·7	4·6	—	—	9·1	7·4	5·11	6·36	5·1	7·87	5·35
Phleum pratense	9·80	6·40	1·20	T.	2·4	8·4	—	—	—	—	5·2	1·0	1·14	2·53	7·6	T.	1·0
Poa spp.*	3·6	T.	T.	—	—	—	—	—	—	—	—	—	—	—	T.	1·06	T.
Agrostis palustris, Huds.	—	—	—	—	—	T.	—	—	—	—	—	—	—	—	—	—	—
Trifolium pratense	34·25	34·20	27·70	29·0	9·5	20·9	28·8	43·8	—	—	30·8	34·50	9·09	30·33	21·6	19·66	16·07
repens	—	—	—	—	—	T.	—	—	—	—	—	—	—	—	—	—	—
hybridium	—	—	T.	T.	T.	1·7	—	—	—	—	—	—	—	—	—	—	—
minus	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1·0	—	—
Medicago lupulina	5·05	6·90	19·70	7·5	3·0	12·6	4·4	5·3	—	—	5·5	9·8	32·39	—	35·3	7·24	7·38
Poterium sanguisorba	—	—	—	—	—	—	—	—	—	—	—	10·8	—	—	—	—	—
Cichorium intybus	—	—	—	—	—	—	—	—	—	—	—	1·0	—	—	—	—	—
Weeds	7·90	3·0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Onobrychis sativa	—	—	—	—	—	—	—	—	—	—	—	4·2	—	—	—	—	—
Plantago lanceolata	—	—	—	—	—	—	—	—	—	—	—	—	5·11	—	—	—	—

* Chiefly *P. trivialis.*

Table V. 1925.

Analysis of chief ingredients of plots. Percentage by weight of plants present in March cut.

Species	1*a*	1*b*	2*a*	2*b*	3*a*	3*b*	4*a*	4*b*	5*a*	5*b*	6*a*	6*b*	7*a*	7*b*	16*b*	17*a*	17*b*
Festuca {*elatior* / *pratensis*	—	—	—	—	—	—	—	—	—	6·42	—	—	—	—	—	—	—
Lolium perenne	13·14	1·38	7·14	15·91	9·56	9·83	29·43	10·28	54·68	6·0	38·13	6·37	23·81	23·68	40·49	4·18	37·37
italicum	67·81	85·76	69·93	66·06	65·55	78·47	40·52	68·95	5·50	72·91	9·69	54·48	45·29	10·87	6·72	89·30	9·08
Dactylis glomerata	4·04	T.	7·33	8·26	13·80	2·44	14·07	15·74	20·84	4·49	31·23	1·0	7·04	43·51	36·64	3·0	20·23
Phleum pratense	1·0	T.	1·94	2·0	1·0	1·0	2·39	T.	1·0	3·10	—	1·0	2·51	—	—	—	1·0
Poa spp.*	1·35	1·0	1·0	1·0	—	—	—	—	—	—	1·0	—	4·52	2·79	1·34	—	4·63
Agrostis palustris, Huds.	1·7	—	T.	1·0	T.	1·22	T.	1·0	—	—	1·67	1·56	T.	T.	1·0	1·0	11·45
Holcus lanatus	—	1·38	—	—	T.	T.	—	—	—	—	—	T.	—	—	—	—	—
Trifolium pratense	10·16	9·74	12·47	5·14	8·96	6·90	12·90	3·66	13·42	5·60	16·27	7·68	14·22	18·71	12·28	1·96	11·67
repens	—	—	—	—	—	—	—	T.	T.	—	1·0	—	T.	T.	T.	T.	—
minus	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	T.
Poterium sanguisorba	—	—	—	—	—	—	—	—	—	—	—	22·94	—	—	—	—	—
Cichorium intybus	—	—	—	—	—	—	—	—	—	—	—	3·29	—	—	—	—	—
Onobrychis sativa	—	—	—	—	—	—	—	—	—	—	—	T.	—	—	—	—	—

* Chiefly *P. trivialis.*

Table VI. 1926.

Analysis of chief ingredients of plots. Percentage by weight of plants present in June cut.

Species	1*a*	1*b*	2*a*	2*b*	3*a*	3*b*	4*a*	4*b*	5*a*	5*b*	6*a*	6*b*	7*a*	7*b*	16*b*	17*a*	17*b*
Festuca {*elatior* / *pratensis*	—	—	—	—	1·50	3·58	—	—	—	21·89	—	—	—	—	—	—	—
Lolium perenne	32·98	1·41	16·44	11·61	13·02	17·17	11·88	13·03	18·87	8·15	12·63	12·83	21·47	17·0	31·67	8·81	18·81
italicum	T.	34·42	7·56	3·88	5·28	3·95	—	3·87	—	1·66	—	3·42	2·31	—	9·01	1·0	T.
Dactylis glomerata	35·47	14·57	43·33	48·70	54·74	48·34	59·92	56·43	49·35	18·45	63·98	9·49	53·48	41·10	29·75	38·65	22·63
Phleum pratense	4·8	9·16	15·02	9·56	5·98	14·62	6·52	10·51	17·23	14·11	10·21	8·05	5·86	18·76	13·87	10·91	4·45
Poa spp.*	4·63	T.	1·92	2·50	0·80	2·68	—	0·72	T.	2·72	T.	2·45	T.	1·32	1·25	T.	T.
Agrostis palustris, Huds.	T.	1·03	3·16	1·88	T.	1·05	—	T.	T.	T.	T.	T.	T.	T.	T.	1·14	4·38
Holcus lanatus	T.	2·84	—	—	—	2·73	—	—	—	1·37	—	—	—	—	—	—	—
Trifolium pratense	10·31	8·53	6·97	12·17	15·66	2·0	16·62	12·69	21·02	29·97	10·24	18·92	6·15	16·77	10·08	38·06	47·18
repens	2·98	2·08	T.	1·21	T.	2·18	—	1·52	T.	T.	—	T.	T.	1·52	1·12	T.	T.
hybridium	—	T.	—	—	—	—	—	—	—	—	—	1·8	—	—	—	—	—
minus	—	3·56	—	—	—	—	—	—	—	—	—	—	—	1·27	1·0	T.	1·11
Medicago lupulina	T.	6·33	—	1·12	T.	T.	—	—	—	—	—	—	—	—	—	—	—
Poterium sanguisorba	—	—	—	—	—	—	—	—	—	—	—	21·75	—	—	—	—	—
Cichorium intybus	—	—	—	—	—	—	—	—	—	—	—	8·01	—	—	—	—	—
Ranunculus repens	—	14·35	—	2·95	1·69	5·65	1·96	—	—	—	—	—	—	—	—	—	—
Cerastium spp.	3·89	—	3·30	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Rumex spp.	—	—	—	2·59	—	—	—	—	—	—	—	—	—	—	—	—	—
Weeds	—	—	—	—	—	6·0	—	—	—	—	—	—	3·4	—	—	—	—
Onobrychis sativa	—	—	—	—	—	—	—	—	—	—	—	9·59	—	—	—	—	—
Plantago lanceolata	—	—	—	—	—	—	—	—	—	—	—	—	2·51	—	1·25	—	—

* Chiefly *P. trivialis.*

Table VII. 1927.

Analysis of chief ingredients of plots. Percentage by weight of plants present in May cut.

Species	1*a*	1*b*	2*a*	2*b*	3*a*	3*b*	4*a*	4*b*	5*a*	5*b*	6*a*	6*b*	7*a*	7*b*	16*b*	17*a*	17*b*
Festuca {*elatior* / *pratensis*	—	—	—	—	3·91	—	—	—	—	33·86	—	1·66	—	—	—	—	—
Lolium perenne	16·38	16·59	8·93	8·92	11·16	7·59	10·47	18·18	17·63	7·14	12·15	11·69	15·23	31·83	29·32	18·28	27·48
italicum	5·00	18·63	3·29	1·97	—	3·0	T.	5·27	—	2·01	0·98	1·77	1·13	—	1·63	3·46	5·89
Dactylis glomerata	57·20	19·94	50·05	53·73	66·89	47·93	45·05	58·48	45·21	20·84	67·97	5·91	67·11	25·89	30·56	35·36	37·01
Phleum pratense	—	7·09	4·86	—	—	—	—	1·22	—	—	—	—	—	—	—	—	—
Poa spp.*	1·11	3·67	—	—	—	14·06	—	—	1·30	—	—	—	1·13	7·51	2·79	2·26	2·13
Agrostis palustris, Huds.	5·74	1·0	15·75	11·92	2·5	T.	6·48	2·60	6·29	4·81	1·39	2·51	2·89	3·82	3·85	9·07	9·40
Holcus lanatus	—	1·59	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1·16
Trifolium pratense	5·22	6·14	7·03	6·03	8·53	4·56	24·65	T.	9·69	2·54	11·49	5·66	T.	1·71	13·44	14·57	8·85
repens	4·70	T.	2·35	7·93	1·09	2·39	T.	2·01	3·05	2·42	3·02	5·0	T.	9·13	10·34	8·12	3·13
minus	1·0	1·28	3·35	—	—	—	—	—	1·54	—	—	—	—	1·53	—	4·76	—
Medicago lupulina	—	—	—	—	—	—	—	—	5·90	6·21	—	—	—	—	—	—	—
Poterium sanguisorba	—	—	—	—	—	—	—	—	—	—	—	35·55	—	—	—	—	—
Cichorium intybus	—	—	—	—	—	—	—	—	—	—	—	6·53	—	—	—	—	—
Ranunculus repens	—	22·16	—	2·55	1·21	2·39	—	—	—	—	—	—	—	—	—	—	—
Cerastium spp.	1·18	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Carduus arvensis	—	—	1·48	—	1·44	6·92	—	—	—	—	—	—	7·22	5·40	—	—	—
Rumex spp.	—	—	—	—	—	—	9·40	5·09	5·47	13·47	—	—	—	—	—	—	—
Weeds	6·0	—	—	—	—	—	—	6·0	4·92	—	—	—	—	6·0	—	—	—
Plantago lanceolata	—	—	—	—	—	—	—	—	—	4·0	—	4·0	—	—	—	—	—
Onobrychis sativa	—	—	—	—	—	—	—	—	—	—	—	14·98	—	—	—	—	—

* Chiefly *P. trivialis*.

STUDIES ON *OSCINELLA FRIT* LINN.

COMPARATIVE RECORDS OF OAT GRAIN INFESTATION IN SWEDEN DURING THE YEAR 1927, TOGETHER WITH A NOTE ON STERILITY OR "BLINDNESS" OF GRAIN

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A RECENT publication (2), which indicated the existence of considerable variation between oat varieties in relation to their resistance to attack by the frit fly in Sweden, summarised data relating to the attack on the shoots during spring. It was not possible to include therein data relating to any differences in resistance which were likely to be exhibited by the grain of different varieties, because the analysis of seed for determination of extent of infestation is a laborious process.

This note should, therefore, be considered as a supplement to the above-mentioned publication, and for this reason a repetition of descriptive matter is superfluous. The method of sampling adopted was the same as that used during similar previous investigations (1). Over 80,000 seed from 313 lots were examined for infestation and sterility, the minimum, mean and maximum numbers of seeds per sample being, in the earlier sown series, 142, 408 and 701; in the later sown series, 101, 185 and 361 respectively.

The percentage infestation and the percentage sterility for each variety for first and second sowings, together with their respective standard errors, where $\epsilon = \sqrt{\frac{P(100-P)}{n}}$, P representing the percentage value and n the number of individuals per sample, are shown in Table I.

These data have also been subjected to statistical analysis by the method of Engeldow and Yule (3). The standard errors of the mean differences in percentage infestation and percentage sterility between any two varieties, for each of the series, are shown in Table II.

The figures in the second and fourth columns indicate the order of the differences between any two mean percentages which may be considered to differ significantly (100 to 1 chance), and thus any two values within a series in Table I may be compared readily. These records are

Table I.

No.	Variety in order of sowing	% infestation: First sowing	% infestation: Second sowing	% sterility: First sowing	% sterility: Second sowing
1.	King	7·8 ± 0·8	25·1 ± 1·4	1·5 ± 0·3	2·9 ± 0·5
2.	Star	6·9 ± 0·8	22·8 ± 1·3	2·2 ± 0·5	3·9 ± 0·6
3.	Victory	5·0 ± 0·5	18·9 ± 1·2	0·7 ± 0·2	3·4 ± 0·5
4.	Golden Rain ...	3·9 ± 0·6	19·5 ± 1·1	2·1 ± 0·4	2·7 ± 0·4
5.	Golden Rain II ...	5·7 ± 0·6	17·7 ± 1·0	1·5 ± 0·3	3·9 ± 0·5
6.	Ligowo	6·8 ± 0·8	22·5 ± 1·3	2·6 ± 0·5	2·2 ± 0·5
7.	Lochows × Victory	3·6 ± 0·5	21·7 ± 1·1	0·8 ± 0·2	2·2 ± 0·4
8.	Lochows	2·7 ± 0·5	17·1 ± 1·1	0·8 ± 0·2	2·7 ± 0·5
9.	Lüneburger Kley	5·2 ± 0·6	18·0 ± 1·1	0·6 ± 0·2	1·4 ± 0·4
10.	Leutewitzer ...	1·9 ± 0·4	13·2 ± 0·9	0·9 ± 0·3	1·6 ± 0·3
11.	Echo	4·3 ± 0·6	19·1 ± 1·1	2·4 ± 0·4	4·3 ± 0·6
12.	Early	3·9 ± 0·5	18·2 ± 1·2	0·7 ± 0·2	2·2 ± 0·4
13.	Victory	6·1 ± 0·6	20·0 ± 1·2	1·1 ± 0·3	3·5 ± 0·6
14.	White Yeoman ...	4·4 ± 0·7	17·4 ± 1·1	2·4 ± 0·6	4·2 ± 0·6
15.	Dala	2·5 ± 0·3	14·7 ± 1·0	3·1 ± 0·4	6·9 ± 0·7
16.	Gophers	5·6 ± 0·6	8·3 ± 0·8	1·5 ± 0·4	1·5 ± 0·3
17.	Kyto	5·2 ± 0·6	11·0 ± 0·9	1·4 ± 0·3	6·7 ± 0·8
18.	Spet	9·3 ± 0·8	29·0 ± 1·4	2·3 ± 0·4	6·8 ± 0·8
19.	Hede	7·0 ± 0·7	30·7 ± 1·4	2·3 ± 0·4	7·0 ± 0·8
20.	Summer	5·3 ± 0·6	12·4 ± 1·0	3·1 ± 0·5	3·3 ± 0·5
21.	Black Bell II ...	6·2 ± 0·6	20·8 ± 1·2	2·5 ± 0·4	7·6 ± 0·8
22.	Engelbrekt ...	5·1 ± 0·6	21·4 ± 1·3	4·2 ± 0·6	5·6 ± 0·7
23.	Great Mogul ...	8·9 ± 0·8	23·3 ± 1·2	5·6 ± 0·6	6·3 ± 0·7
24.	Argus	6·4 ± 0·8	24·1 ± 1·4	1·3 ± 0·3	4·2 ± 0·6
25.	Roslags	7·0 ± 0·7	22·7 ± 1·2	1·9 ± 0·4	9·0 ± 0·8
26.	Victory	7·2 ± 0·7	22·4 ± 1·2	1·6 ± 0·3	4·1 ± 0·5
27.	Black Tartar ...	4·8 ± 0·6	26·5 ± 1·3	2·8 ± 0·4	3·7 ± 0·6
28.	Black Supreme ...	11·8 ± 1·1	22·5 ± 1·5	7·0 ± 0·9	11·6 ± 1·2
29.	Orion	5·0 ± 0·5	10·0 ± 0·9	9·8 ± 0·7	11·9 ± 0·9
30.	Mesdag	5·6 ± 0·7	9·0 ± 1·0	2·6 ± 0·5	5·6 ± 0·8
31.	Sandy	6·8 ± 0·8	15·0 ± 1·1	3·5 ± 0·6	9·9 ± 0·9
32.	Tam Finlay ...	9·1 ± 1·3	14·5 ± 1·3	4·9 ± 1·0	20·2 ± 1·5
33.	Kent Berlie ...	9·9 ± 1·0	16·8 ± 1·1	2·7 ± 0·5	17·0 ± 1·1
34.	*Avena fatua* ...	6·7 ± 0·7	24·4 ± 1·7	6·0 ± 0·7	18·6 ± 1·5

Table II.

	Infestation: Standard error of mean difference	Infestation: Standard error × 2·57	Sterility: Standard error of mean difference	Sterility: Standard error × 2·57
First sowing	1·11	2·85	0·77	2·06
Second sowing	1·82	4·68	1·48	3·82

comparative only in so far as the grain was the product of varieties sown at the same time. The different varieties, shooting at different times, were subject to varying intensities of attack, according to the variation in the fly prevalence (2). Detailed acquaintance with the growth of each variety is required to enable one to arrange for simultaneous shooting in the field.

In this case, in the first sowing, the earliest varieties commenced shooting on July 12th, just prior to the commencement of the emergence

Table III.

Relative time of shooting.

		Early		Normal		Late	
No.	Variety	1	2	3	4	5	6
1.	King	·	·	·	·	×	·
2.	Star	·	·	×	·	·	·
3.	Victory	·	·	·	×	·	·
4.	Golden Rain	·	·	×	·	·	·
5.	Golden Rain II	·	·	·	×	·	·
6.	Ligowo	×	·	·	·	·	·
7.	Lochows × Victory	·	·	×	·	·	·
8.	Lochows	·	·	×	·	·	·
9.	Lüneburger Kley	·	·	×	·	·	·
10.	Leutewitzer	·	·	·	·	×	·
11.	Echo	·	·	·	·	·	×
12.	Early	·	·	×	·	·	·
13.	Victory	·	·	·	×	·	·
14.	White Yeoman	·	·	·	×	·	·
15.	Dala	·	×	·	·	·	·
16.	Gophers	×	·	·	·	·	·
17.	Kyto	×	·	·	·	·	·
18.	Spet	·	·	·	×	·	·
19.	Hede	·	·	·	·	·	×
20.	Summer	×	·	·	·	·	·
21.	Black Bell II	·	·	·	·	×	·
22.	Engelbrekt	·	×	·	·	·	·
23.	Great Mogul	·	·	·	·	·	×
24.	Argus	·	·	·	×	·	·
25.	Roslags	·	·	·	·	×	·
26.	Victory	·	·	·	×	·	·
27.	Black Tartar	·	·	·	·	×	·
28.	Black Supreme	·	·	·	×	·	·
29.	Orion	×	·	·	·	·	·
30.	Mesdag	×	·	·	·	·	·
31.	Sandy	·	·	·	·	·	×
32.	Tam Finlay	·	·	·	·	·	×
33.	Kent Berlie	·	·	·	·	·	×
34.	*Avena fatua*	·	·	·	×	·	·

period of the summer generation of flies, and the majority of the panicles had emerged by July 23rd, *i.e.* before the fly prevalence was very great. In the second sowing, except for Mesdag, which produced all its panicles between July 15th and 19th, shooting commenced on July 17th and extended over a period of about 17 days, well within the period of fly prevalence.

It is only necessary to point out, as is shown by comparison of Tables I and III, that all except one of the very early shooting varieties, namely Gophers, Kyto, Summer, Orion and Mesdag, suffered least infestation, the exception being Ligowo, which may therefore be more susceptible. The medium groups (3–4) suffered to much the same extent, Spet being the worst. Leutewitzer, a late form suffering lightly, may be resistant. Of the very late group, Sandy, Finlay and Kent Berlie suffered lightly, late shooting suggesting itself as the probable explanation, but Hede, another very late type, was more heavily infested than any other variety.

It is interesting to note that Summer holds a high position as regards both shoot and grain infestations, because this variety has been used extensively for hybridisation.

Sterility of grain.

The numbers of "blind" or sterile grains were recorded for each sample during the examination of the grain for infestation, because it has been suggested frequently that blindness is due primarily to frit fly. There being but one season's data, only a tentative interpretation is made, but these data appear worthy of record if only for their suggestiveness of the necessity for more detailed investigation with this special point in view.

Table IV.

Correlation coefficients.

Sterility.	
Series I–III and IV–IX	$+0{\cdot}63 \pm 0{\cdot}07$
Ditto, omitting nos. 32–34 which matured badly	$+0{\cdot}72 \pm 0{\cdot}06$
Infestation.	
Series I–III and IV–IX	$+0{\cdot}36 \pm 0{\cdot}10$
Sterility × infestation.	
Series I–III	$+0{\cdot}38 \pm 0{\cdot}10$
IV–IX	$-0{\cdot}02$
Oxford, 1924 data	$-0{\cdot}28 \pm 0{\cdot}19$
1923 data	$+0{\cdot}56 \pm 0{\cdot}13$
Harpenden, 1923 data	$+0{\cdot}11 \pm 0{\cdot}19$

Certain correlation coefficients between the values shown in Table I for the first and second sowings have been calculated and are shown in Table IV, together with three coefficients calculated from data collected during previous years.

Sterility or "blindness" of grain is possibly due to the operation of several factors, which may vary in intensity from year to year. Suggested factors have included weather conditions at time of fertilisation, the action of insects such as frit fly or thrips on the ovule and the immature grain, the action of mites or the possible influence of variety.

If weather conditions were responsible for sterility, a correlation would not result unless the conditions were identical at the corresponding periods of fertilisation and, under such conditions, varieties shooting during the same periods should show sterility to similar extents. But Ligowo, Gophers and Summer were much less sterile than Orion, which flowered during the same period, while amongst the late varieties Echo, Hede, Great Mogul and Sandy suffered much less than Tam Finlay and Kent Berlie. *Avena fatua* in the normal group suffered heavily.

If frit fly were responsible then percentage sterility should correlate with percentage infestation. Reference to Table IV shows that in none of the five cases quoted does any correlation exist, and therefore sterility of grain cannot be said to be due, to any appreciable extent, to the frit fly.

On the other hand, the correlation between the two series of figures showing percentage sterility is very decided and indicates that degree of sterility may be a varietal character. The coefficient is not high, showing that other factors are also involved.

SUMMARY.

Data, collected in Sweden during the year 1927, indicate the existence of considerable variation in extent of infestation of the grain of different varieties, sown at the same time. Also they suggest that sterility or "blindness" of grain may be a varietal character.

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SIGNIFICANT VARIABLES IN THE BLOWFLY ENVIRONMENT[1]

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(With 1 Text-figure.)

I. General considerations.

The numerical abundance of a living insect, in a measured volume of the surrounding medium, at the end of a measured time period during which it has been subjected to the influence of a combination of environmental factors each of known quantitative value, may be expressed as

$$P_t = \frac{P_o + P_o . Z^n}{R^n},$$

where P_t is the required value of population density; P_o is the value of initial population density; Z is the biotic potential or mean maximum rate of reproduction under the conditions given, and a product of the mean decimal proportion of mature females in the initial population by the mean number of births per female; n is the number of generations occurring during the time period; and R is the mean total value of environmental resistance to the biotic potential of each generation, a quantitative expression of the tendency of an environment to restrict potential abundance.

Since, however, the successive generations may differ from one another in mean sex ratio, in mean birth rate, and in mean death rate, the formula is more correctly expressed as

$$P_t = \frac{P_o . Z_1 . Z_2 \dots Z_n}{R_1 . R_2 \dots R_n},$$

where $Z_1 \dots Z_n$ are the biotic potential values of the successive generations and $R_1 \dots R_n$ are the total resistance values of the environments of the successive generations.

If the pre-maturation period of the life cycle be shorter than the post-maturation period, the generations will overlap one another, and if the

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environment remains uniform within narrow limits and is favourable to the organism, the value of R will remain approximately constant and the rate of increase (r) will, after a number of generations, become practically constant, and calculable from the formula

$$r = \frac{\log P_o - \log P_t}{\log_e n},$$

where P_o and P_t are the adult population densities at the commencement and termination of any given number (n) of generations, and e is a constant of value 2·71828.

Eventually, however, since the environment is finite, the population density should reach a maximum value for that particular value of environmental volume. As it approaches this maximum value, the values of Z and R will approach one another in value, partly through a decrease in fecundity of the organism, partly through an increase in its rate of mortality, induced by crowding, so that the population density will eventually remain relatively stable at a value approximating to the maximum value for that size of environment.

That is to say, the ideal curve expressing the incidence of abundance of an adult insect in a restricted environment is a logistic curve, and the number of generations which must elapse before the upper asymptote of the curve is reached will depend, in the first place, upon the size of the environment, since the value of maximum population density varies as the square of the volume of the environment (Pearl (12)); in the second place, upon the extent to which the environmental factors fluctuate beyond the range of values optimum for the organism; and in the third place, upon the value of the organism's biotic potential.

Such an equilibrium of adult population density, within a restricted environment, under highly favourable environmental conditions, and for organisms possessing a high biotic potential, has been demonstrated by Pearl (12) for *Drosophila melanogaster*, and by Chapman (3) for *Tribolium confusum*.

Now, in the case of a blowfly species, the pre-maturation period of the life cycle under temperate climatic conditions is usually longer than the post-maturation period, and the extent to which successive generations overlap one another is slight; the annual sequence of generations is short, comprising, in Great Britain, for example, only four generations in the case of *Calliphora* and *Lucilia*; among the four generations there is one whose prepupal stage is abnormally prolonged; the significant environmental factors have each a wide range of variability and on the whole are antagonistic to the biotic potential of the insect; further, the adult insect

is winged and strongly mobile so that its environment is practically unrestricted.

A curve expressing the incidence of abundance in a locality of a succession of blowfly generations is in fact a polymodal curve whose modes correspond to the maxima of abundance of the successive generations (Wardle (17)), and it is probable that, in the case of a blowfly species, an asymptotic condition of population density is reached only rarely, and only then in localities where there is an abnormal plenitude of oviposition media, chemotropically attractive to the flies, and only for a brief period of time.

The environment of an insect is a complex of factors, that is to say, of unit sources of influence, and the value of each factor will fluctuate normally over a certain range of variability. For each factor there is a *range of effective values* favourable to the organism in that they permit its continued existence and within this effective range, again, there is a *range of optimum values* favourable to maximum development and comfort. The significance of an environmental factor as regards its influence upon potential abundance will depend upon the ratio between the range of variability of the factor, under normal field conditions, and the range of values which are optimum for the organism under consideration. If the width of the latter range exceeds that of the former, the factor will be without significance for the organism, but should the reverse condition be the case, or the width of the optimum range be narrow as compared with that of the normal range of variability, then the factor will be a *limiting factor* to potential abundance; to use the preferable nomenclature of Allen (1) it will be a *significant variable*.

There are three types of significant variable in the blowfly environment, namely:

(i) *Primary variables*, which influence the biotic potential and the duration values of life cycle stages;

(ii) *Secondary variables*, which influence the biotic potential but not the duration values of life cycle stages;

(iii) *Tertiary variables*, which influence behaviour but not biotic potential nor duration values.

A single factor may act as a significant variable independently of other factors, but usually the significant variables in the environment are associated in groups which, in their resistance to the biotic potential, act like single variables.

The value of R for any sum total of variables will have a minimum value of unity since it is lowest when P_t equals $P_o . Z_n$. Since also R is the

product of the environmental resistance values $r_1 \ldots r_n$ of the component factors or factors groups in the sum total, these values will also each have a minimum value of unity.

For any measured factor value the resistance value will be $P_o . Z_n$ divided by P_t, but this value will vary as the factor value varies, and will vary for different periods of the life cycle. The whole life cycle in fact may be integrated into a series of monecological phases each characterised by a definite series of resistance values (r) and duration values (d) corresponding to the measured variations of each significant variable in the environment.

Quantitative measurements of the r and d values for any significant variable can only be obtained in an environment in which the factor can be varied to a measurable extent, whilst other factors remain within the range of optimum values, that is to say, of values whose resistances approach unity. Before values of r and d can be obtained, therefore, for each monecological phase of the life cycle, it is necessary to establish for each phase an optimum environment, an environment composed of primary variables, each of such a value that within the environment P_t equals $P_o . Z$ and d is at a minimum.

Within such an optimum environment, the variation of any one factor to a measured extent, or the introduction of other factors of known value, will permit a series of r and d values to be obtained which will correspond to a series of variations of any primary or secondary variable from the optimum. From the series of r and d values, quantitative values of R and D and n can be computed.

The data presented here concern the establishment of the optimum values of temperature and humidity, regarded as primary significant variables, for the pre-imaginal stages of *Lucilia sericata* Meigen, and the influence of ultra- and infra-optimum variations of these factors upon the biotic potential and duration value of each pre-imaginal stage.

II. The pre-imaginal environment.

The pre-imaginal life cycle of *Lucilia sericata* comprises the stages of egg, larva, prepupa and pupa. For experimental purposes these can be regarded as monecological phases. Theoretically greater experimental accuracy would be obtainable by regarding each stage as a series of growth phases, but in practice the difficulty of differentiating such phases one from another would detract from the accuracy of the results.

This period of the life cycle requires three media, namely, atmosphere, protide and soil.

The egg stage is spent upon the aphotic surface of decomposing albumin or globulin protide material, but actually it may be regarded as an atmospheric stage, since it can develop equally well away from the oviposition medium. The larval stage is spent in a semi-buried position within protide media. The prepupal and pupal stages are spent normally within the surface layers of the ground.

The primary significant variables would appear to be **temperature, humidity** and **oxygen**, although the influence of the latter factor remains to be investigated; and in particular the temperature and humidity of atmosphere and soil.

The outstanding secondary variables are **accessible protide** and certain **biotic factors**. Accessible protide may be defined as the mass of oviposition medium available per mature female.

Taking the mean mass of the newly hatched larva as 0·00015 gm. and that of the full fed larva as 0·05 gm., the utmost minimum larval requirement of food medium will be approximately 0·05 gm. Its actual requirement will in fact be higher than this. Assuming the maximum number of eggs produced per female to be 900, it would appear that the utmost minimum requirement of accessible protide will be approximately 45 gm. per ovipositing female. Actually, owing to the fact that only a limited area of the surface of a mass of suitable oviposition medium can be utilised for egg deposition, the female requirement of accessible protide is considerably higher than this value.

When blowflies are abundant, the number of eggs which is deposited upon a suitable mass of oviposition medium is very great, and may be in excess of the number of larvae for which the medium can afford space and sustenance. Thus, at Manchester, England, where *Calliphora* (71 per cent.) and *Lucilia* (21 per cent.) are the predominant blowfly genera, the number which can be reared from a small unskinned mammal, such as a rabbit with an unconsumable proportion of 25 per cent., is approximately four flies per gm. of medium consumed, despite the deposition on the carcase of an estimated number of 10,000–15,000 eggs. At St Paul, Minnesota, where *Lucilia* (52 per cent.) and *Phormia* (43 per cent.) predominate, the number of flies which issue from a small piece of ox liver whose non-consumable proportion is 10–20 per cent., is approximately six per gm. of medium consumed, although the number of eggs deposited will rarely be less than 500. Smit (15) records in South Africa the emergence of 1451 individuals of *Chrysomyia albiceps* from an exposed sheep carcase weighing 36 lb., an average of only one fly per gm. of medium.

Under field conditions of imaginal abundance, even a ratio of 45 gm.

of medium per female is rarely available, and despite the strongly developed chemotropic propensities and the wide range of flight of blowfly imagos, there is under normal field conditions a high degree of competition among ovipositing females and among newly hatched larvae.

The female fly may be forestalled in securing a position upon a suitable oviposition mass by more chemotropically sensitive individuals of the same or of other species, or, if successful in securing a foothold may be jostled therefrom by more aggressive individuals. Lownes(9) has asserted that the female *Calliphora erythrocephala* consciously adapts the size of her egg cluster to the available oviposition surface of the medium. Such conscious limitation does not occur in the case of *Lucilia sericata.* Even when the only available surface of the medium is covered with egg clusters, females will continue to add their eggs to the pile, or will even deposit clusters upon the soil in the vicinity of the medium. The size of the egg cluster is determined chiefly by the degree of freedom from disturbance that the female can secure. The fly is shy and readily dislodged from the medium by other flies, unlike the more phlegmatic *Calliphora erythrocephala*, which will continue steadily to deposit eggs at the rate of one per second, oblivious of the needle of the observer which removes each egg as soon as laid.

It is possible that such competition for suitable oviposition facilities may tend to provoke in temperamental species of blowfly a retention of ripe eggs in the oviducts and so intensify the desire to oviposit. It may thus provide a partial explanation of two phenomena of calliphorid biology, namely, a tendency towards viviparity, occasional in most blowfly species, but habitual in some, and a tendency towards the use of living animal tissue as an oviposition medium. It seems not without significance that in the sheep-maggot districts of Texas there is a competition between *Lucilia sericata* and *Cochliomyia macellaria*; that in South Africa, the sheep-maggot season (November to April) coincides with the maxima of abundance of *Lucilia sericata* and *Chrysomyia chloropyga* (Smit and Du Plessis(16)); that in the sheep-maggot districts of Great Britain there is competition between *Lucilia sericata* and five other blowfly species; in Australia, competition with eleven other species; in New Zealand, competition with five other species. Competition, however, can be only one of several factors which induce the sheep-attacking habit, since there are many localities where the habit is uncommon despite the abundance of blowflies.

The competition between newly hatched larvae to secure nutritional facilities is intensified by factors which desiccate the medium and make

portions of it unsuitable for larval nutrition, by unconsumable constituents such as fats and scleroproteins, or by obstacles to the entrance of larvae to the medium. Thus, with a hairy skinned carcase, entrance is more readily effected at places such as groin and axilla, edges of natural orifices, where the skin is relatively thin and hairless; with a fish, entrance is more readily effected via the orbit or the gills; at such points the female fly instinctively oviposits, stimulated thereto possibly by conditions of surface humidity, and relatively enormous masses of eggs may accumulate there. When the egg masses commence to hatch, the competition to gain admission to the medium is severe. Under conditions of darkness there is a considerable migration of young larvae away from the medium, and large numbers of such migrants perish in their search for other nutritional facilities.

With regard to the significance of biotic factors such as predators, parasites and pathogenic organisms towards potential abundance, the evidence is scanty. Graham-Smith (5) has recorded at Cambridge, England, the occurrence of 61 per cent. of autumn pupae of *Calliphora erythrocephala* parasitised by the Braconid Hymenopteron *Alysia manducator* under shade conditions, and of 71 per cent. of spring pupae parasitised by the Chalcid Hymenopteron *Melittobia acasta* under sunshine conditions. Although these parasites also attack the species of *Lucilia* it would seem doubtful whether the Chalcid parasites—*Melittobia acasta* and *Mormoniella tripennis* (*Nasonia brevicornis*)—at any rate, can offer a high degree of resistance to potential abundance, since they usually attack prepupae uncovered by soil, and such prepupae are relatively infrequent.

James (8) asserts that Cynipid parasites of the larval stages of *Lucilia sericata* and *Calliphora erythrocephala*, such as *Figites anthomyiarum* Bch. and *Kleidotoma marshalli* Mshl. are responsible in England for a diminution in abundance of 30 per cent.; that is to say, their resistance value is 1·43.

Among other secondary significant variables the proportion of clay in the soil is of some degree of importance, since a heavy clay soil may compact under the influence of rain and impede the emergence of imagos from the ground, whereas a sandy soil will favour such emergence.

Air movements and the precipitation of atmospheric humidity are also secondary significant variables, but their influence is an indirect one brought about by the effect upon atmospheric temperature and humidity.

The more important tertiary variables are those of **light** and **temperature**. Neither the intensity nor the wave-length of light appears to

influence the potential abundance nor the duration of the life cycle, but light has a marked influence upon adult and larval behaviour. Blowflies do not copulate nor oviposit in the complete absence of light. The ovipositing females of *Lucilia* are attracted to media exposed to bright sunshine, although they actually oviposit upon the shaded portions of the medium's surface. *Lucilia sericata* oviposits in Minnesota usually between the hours of 11.00 and 14.00 when the sunlight is at its maximum daily intensity. *Lucilia caesar* is less sensitive to light, and will oviposit when the sky is overcast. *Lucilia sylvarum* will oviposit in late afternoon when the intensity of light is low and when atmospheric temperatures may be low, an interesting habit in view of the suggestion that this species is synonymous with the European *bufonivora* which is known in Europe to oviposit within the nostrils of toads.

The effect of light upon larval and prepupal behaviour has been discussed by Herms (6), who has shown that feeding larvae of *Lucilia caesar* react instantaneously and negatively to daylight, but react more slowly and positively to artificial light of all visible wave-lengths, unless given a choice of colours, when they show a preference for wave-lengths of 0·535–0·586 (yellow). Since these reactions are stronger than the positive reactions to food, they may be detrimental in that they tend to separate the larva from its food medium. On the other hand, in what this observer terms "migrated larvae," referring probably to prepupae, the negative response to daylight is slower and more pronounced, and there is a negative reaction to artificial light. They are most pronouncedly negative to the blue end of the spectrum (0·422–0·492).

The influence of temperature upon larval behaviour has been discussed by Miller (10). The rate of locomotion of larvae of *Lucilia sericata* varies directly with temperature between 20° C. and 40° C.; the rate of contraction increases directly with temperature between 0° C. and 45° C.; the number of contraction waves made by a larva in travelling a given distance such as 10 cm. is a constant between the temperature of 10° C. and 33° C. Below 10° C. the rate of contraction decreases and the rate of locomotion decreases. Above 33° C. the rate of contraction increases, and up to 40° C., to judge from Miller's graphical figures, the rate of locomotion increases.

III. MATERIAL AND METHODS.

Lucilia sericata occurred at St Paul, Minnesota, on the Agricultural College campus, during July, August and September, 1927, in the proportion of 28 per cent. of the blowfly population, competing with *Lucilia*

caesar (24 per cent.), *Lucilia sylvarum* (4 per cent.), *Calliphora erythrocephala* (1 per cent.), and species of *Phormia* and *Protocalliphora* (43 per cent.).

Lucilia sericata may be defined here as a Luciliine fly, answering to the description of Shannon (14), and distinguishable among North American species of *Lucilia* by the possession of a yellow basicosta and three post-acrostichal bristles, as contrasted with the black basicosta and two post-acrostichal bristles of *caesar* and the black basicosta and three post-acrostichal bristles of *sylvarum*. About 5 per cent. of the flies identified as *caesar* had a greyish basicosta, and these may have belonged to the species *australis*.

The material used was obtained from egg clusters deposited in the open on ox liver under conditions of bright sunshine. The moment of deposition was known to $\pm$ 2 minutes. The number of eggs per cluster varied from 50 to 373. Whether more than one cluster can be deposited by an individual was not determined, but as the mean longevity of the female in captivity is three weeks, and as each ovary contains 90 egg tubes, each with five or six potential eggs, it would seem as if more than one cluster can be deposited. According to Babcock and Bennet (2), the allied fly *Cochliomyia macellaria* in Texas can deposit eight clusters each of 40–250 eggs, totalling 10,000 eggs or more, within a period of a week. Probably, however, in the case of *Lucilia sericata*, the degree of competition between females will tend to restrict the potential oviposition capacity.

A portion of each cluster was reared through to the imaginal stage for purposes of identification, at outdoor temperatures fluctuating between 14° C. and 30° C., in a saturated atmosphere and, in the case of prepupal and pupal stages, in a saturated soil medium. Of 110 clusters thus identified, 92 belonged to *sericata*, 12 to *caesar*, 2 to *sylvarum* and 4 to *Calliphora erythrocephala*. No explanation can be offered for the absence of egg clusters of *Phormia* and *Protocalliphora*, despite the numerical abundance of these genera, but it may be noted that only clusters were used that had been deposited in bright sunshine. The remainder of a cluster, if a large one, was separated into batches of approximately 25 eggs, and each batch was transferred to a 50 gm. piece of ox liver in a circular rearing can, 7 cm. in diameter, with a tightly fitting lid.

A series of environments, approximately constant as regards temperature, was obtained in a series of constant temperature cabinets, the values being 37°, 30°, 26°, 20°, 17·5°, 14° and 10° C. The maximum and minimum effective temperatures were not determined with precision,

but appeared to lie between 35° C. and 40° C. and between 4° C. and 6° C. for each stage. As will be shown later, the point at which the temperature axis is cut by the reciprocal of the curve which best fits the mean duration values of the respective life cycle stages, suggests minimum threshold values of 5·6, 5·3, 4·2, and 5·6 for egg larva, prepupa and pupa respectively. Peairs (11) suggests for the larva and pupa of *Lucilia caesar* minimum threshold values of 5·5 and 6·5 respectively.

A saturated atmosphere was obtained by lining the small rearing cans with water-saturated tissue wadding; other values of relative atmospheric humidity were obtained by keeping the cans with lids partly open in desiccators over dilutions of sulphuric acid, or over saturated solutions of salts, of known vapour tension. It is probable that the use of *mass of water vapour per unit of air* instead of relative atmospheric humidity would give greater accuracy of results. A number of egg clusters were reared merely to the moment of larval emergence, and in such cases the relative atmospheric humidity values were obtained by using tightly corked tubes, 9 cm. by 2·5 cm., containing either distilled water or dilutions of sulphuric acid or saturated solutions of salts. The eggs were enclosed in a smaller tube suspended within the larger one, or were floated on rafts of paraffin wax on the surface of the fluid. The values of relative atmospheric humidity employed were 100, 80, 60, 40, 20, 0 per cent., so that eggs were incubated in 42 environments.

The larval stages were reared in ox liver of initial moisture content 25–35 per cent. A ratio of 25 larvae to 50 gm. of medium was used so as to reduce, as far as possible, any resistance to potential abundance induced by spatial competition. Ox liver is not altogether satisfactory as a medium, since its moisture content is influenced by prevailing atmospheric humidity, and there is in addition to the moisture content a non-consumable proportion of 10–20 per cent. An artificial medium would be preferable. It was found impossible to rear larvae in atmospheres whose relative humidity was below 60 per cent. owing to the desiccation of the ox liver and the consequent migration and death of the larvae. Larval stages were reared therefore in 21 environments.

As soon as the full-fed larvae commenced to leave the medium they were transferred to cans containing a layer of soil 1 cm. thick. The soil was a finely sifted sandy type which required, after heating for 2 hours at 100° C., the addition of 40 per cent. of its mass of distilled water to saturate it. The soil moisture values adopted were 100 (saturation), 75, 50, 25 and 0 per cent. (absolute dryness), so that the prepupae and pupae were reared in 35 environments.

The mean sex ratio of the flies reared was 53 : 47 males to females. According to Herms (7), however, the sex ratio of *Lucilia sericata* varies between 28 : 72 and 65 : 35 males to females according to the length of the larval feeding period, starvation raising the rate of mortality among female larvae and so producing an excess of males, a fact which has been noted also by Weidling (18) for *Calliphora erythrocephala*. Taking Herms' figures and estimating the maximum number of eggs per female as 900, the value of Z for *Lucilia sericata* should lie between 315 and 648. The value of n for the number of generations between May and October is 4 in northern latitudes, so that the potential number of individuals at the end of the flight season, May to October, should lie between 315^4 and 648^4 for each female of the emerging generation of spring individuals.

IV. The incubation period.

It is probable that egg development commences before the egg has left the parental oviduct, but the incubation period will be regarded here as commencing with the moment that the egg is completely extruded from the parental cloaca, and terminating with the moment when the larva has completely extricated itself from the shell, an operation which requires a minimum of 2 minutes.

The majority of eggs were incubated in darkness, preliminary experiments having suggested that the incubation period is not affected in duration value by the absence of light. The incubation values for the particular environments used were as follows, the values being given in hours:

	Relative atmospheric humidity		
Temp. °C.	100 %	80 %	60 %
37	7·5– 7·8	—	—
30	9·6–10·4	10·5–11·0	11·5–13·0
26	11·5–12·0	12·5–13·5	14·0–18·0
20	19·5–20·5	21·0–23·0	24·0–26·0
17·5	27·0–28·0	29·0–31·0	33·0–35·0
14	47·0–49·0	51·0–53·0	53·0–56·0
10	88·0–92·0	94·0–100·0	108·0–112·0

At 10° C. there is a tendency for moisture to condense upon the surface of the cluster, and the duration values obtained at this temperature value may be too high.

The two values given for each environment represent the mean values of incubation commencement and incubation termination, respectively, of a number of egg batches. The interval between each pair of values may

be termed the *incubation interval*, and it is found to vary from 20 minutes at high temperatures to 4 hours at low temperatures and low atmospheric humidities. The incubation interval for a small batch of eggs is believed not to differ materially from that of a natural cluster, to judge from data obtained by incubating natural clusters.

No incubation values can be given for atmospheric humidity values lower than 60 per cent., as at such values the mortality among eggs was almost absolute.

If the reciprocal of the mean of each pair of incubation values be plotted the resulting curve appears to be a skew curve. It is simpler, however, to regard the mean incubation values at 14° C. and above as best fitted by a curve of value

$$y = \frac{1}{0{\cdot}117x - 1{\cdot}12},$$

where y denotes the incubation value and x the temperature. The reciprocal of this curve is a straight line which cuts the temperature axis at 9·5, a value obviously too high to represent α, the threshold temperature of egg incubation. The mean incubation values at temperatures below 14° C. are best fitted by a curve of value

$$y = \frac{1}{0{\cdot}06x - 0{\cdot}34},$$

whose reciprocal cuts the temperature axis at 5·6.

It may be suggested, therefore, that the threshold of egg development is around 5·6° C. and that at temperatures above 14° C. there is an acceleration of the velocity of development, associated possibly with the increase in mass of water vapour per unit of saturated air as the temperature rises.

The values of R for the various environments may be expressed as follows:

	Relative atmospheric humidity		
Temp. °C.	100 %	80 %	60 %
37	1·01–1·07	—	—
30	1·01–1·07	1·05–1·11	1·17–1·25
26	1·01–1·07	1·11–1·17	1·17–1·33
20	1·01–1·07	1·11–1·17	1·17–1·33
17·5	1·01–1·07	1·11–1·17	1·36–1·96
14	1·01–1·07	1·11–1·17	1·36–1·96
10	1·01–1·07	1·11–1·17	1·36–2·50

These values, however, are subject to a correction for the resistance induced by experimental handling, a correction which lies between 1·01

and 1·07. That is to say, at temperatures within the effective range and at 100 per cent. relative atmospheric humidity, the resistance to the biotic potential of the egg stage should be at its minimum and P_t should equal P_o.

The mortality among eggs at humidities below 100 per cent. is due to a contraction of the egg shell which kills the contained embryo or prevents the emergence of the formed larva.

V. The larval period.

The larval period lasts from the moment when the emerging larva is free from the egg shell to the moment when it ceases finally to feed.

Restricting the term *larva* to this period of the life cycle, there would appear to be in the case of *Lucilia sericata* two larval stages, namely, a short one terminated within 2 or 3 hours after hatching by a moult, and a longer one lasting from $1\frac{1}{2}$ to $9\frac{1}{2}$ days according to temperature and again terminated by a moult. The so-called third larval stage of other observers is identical with the stage termed here prepupal.

Of the two larval stages, the short preliminary one is largely a non-feeding period spent by the larva in securing an advantageous position within the medium. It is the stage during which, in the field, mortality is high. The second stage is the true feeding period during which, unless interrupted by unfavourable changes in the medium, the larva feeds steadily until it attains the condition termed by entomologists "full fed." These two stages of the larval period will be regarded here as constituting one monecological phase.

The exact moment when the larval period comes to an end is difficult to determine with precision. In the majority of individuals it is marked by the migration of the animal from the medium, but many full fed individuals remain within the medium or migrate into the soil immediately below it.

In the following table of duration values, therefore, although the two values given for each environment represent mean minimum and maximum duration values, all that can be affirmed with safety is that for the particular combinations of temperature and humidity the *majority* of larvae in a batch have duration values lying between the limits given. It is not disputed that many individuals have duration values lying outside these limits.

The duration values are as follows, the periods being given in hours:

Temp. °C.	Relative atmospheric humidity 100 %	80 %	60 %
37	36–60	—	—
30	48–72	48–72	—
26	60–84	48–96	48–120
20	96–120	96–144	108–168
17·5	108–132	108–156	96–192
14	168–192	168–192	168–216
10	240–384	250–370	300–384

At 37° C. and in an atmosphere whose relative humidity is below saturation, the larvae are restless and prone to leave the medium before they are full fed. Similar behaviour is shown at temperatures around 30° C. when the humidity is below 80 per cent. and at all temperatures when the humidity is below 60 per cent.

The data concerning the length of the larval period under such conditions are unreliable and are omitted here. The influence of low atmospheric humidities upon larval behaviour is probably an indirect one and induced by physico-chemical changes in the food medium. Ox liver when in small pieces is very susceptible to desiccation. In the field, where the larvae are feeding within relatively large masses of medium, such behaviour at low atmospheric humidities may not occur.

Variations in atmospheric humidity, providing that the value does not fall below 60 per cent., probably affect only slightly the duration value for a given temperature value, since the animal, owing to its habit of liquefying the food medium, creates its own atmosphere, and lives in an atmosphere approaching saturation.

Changes in atmospheric temperature of less than 10° C. in value do not appear to influence the duration value appreciably, possibly owing to the embedded, semi-insulated position of the larvae in the food medium. Under field conditions there is undoubtedly a high degree of resistance to potential abundance through the failure of first stage larvae to secure a place within the medium. Under conditions of laboratory experiment, however, competition can be reduced to a minimum and the actual resistance to potential abundance should not exceed 1·1, a value which can be ascribed to imperfections of technique and to the tendency of some individuals to remain within the medium and be killed by its subsequent desiccation. In the field, too, there is probably a high degree of resistance to potential abundance induced by parasites and predators of the second larval stage.

Peairs (11) found that, in the case of *Lucilia caesar*, the percentage

of larvae which did not develop further varied from 94·8 per cent. at 6° C., to 11 per cent. at 20° C., to 54 per cent. at 35° C., the humidity value being 100 per cent. Peairs, however, included under the term larva the stage regarded here as prepupal, a stage in which the rate of mortality at all temperatures is admittedly high.

At temperatures between 10° C. and 37° C. and at a relative atmospheric value of 100 per cent., the mean duration values expressed in days are best fitted by a curve of value

$$y = \frac{100}{1{\cdot}6x - 8{\cdot}6},$$

whose reciprocal cuts the temperature axis at 5·3, which may be regarded as approximating to the threshold temperature for larval development.

VI. The prepupal period.

The prepupal stage may be defined as a non-feeding, edaphic, mobile phase of the blowfly life cycle, which intervenes between the second larval moult and the commencement of pupation. It is a stage spent within the soil or close to the soil surface and, within the temperature range of 10–37° C., the chief significant variable in its environment would appear to be the soil moisture content. Any variations of atmospheric humidity can only exert an influence through their indirect effect upon the soil moisture content.

The precipitation of atmospheric humidity as rain may, however, have a directly inimical effect upon potential abundance should the water duty be sufficiently heavy to force the prepupae deeply into the soil or to drown them.

The prepupal duration values for various combinations of atmospheric temperature and soil moisture content are as follows.

In the experiments the soil layer was so shallow that the use of atmospheric temperature values instead of soil temperature values should not appreciably affect the data. The values are expressed in hours.

	Soil moisture content				
Temp. °C.	100 %	75 %	50 %	25 %	0 %
37	80–88	—	48–72	—	—
30	50–130	60–137	60–108	45–82	85–120
26	—	—	72–144	—	—
20	—	—	72–168	—	130–180
17·5	—	—	96–192	—	—
14	—	—	144–240	—	—
10	—	—	264–408	—	—

The gaps in the table indicate where the mortality exceeded 70 per cent. At all combinations of atmospheric temperature and soil moisture content, some prepupae were reared through to the pupal stage, but under conditions of soil saturation and absolute dryness, and to a lesser degree, of 75 per cent. and 25 per cent. of saturation, the percentage mortality was heavy, often in a batch being absolute. Particularly was this the case at temperatures below 30° C.

It is not certain that such a high value of resistance to potential abundance can be attributed altogether to temperature and soil moisture conditions. The occurrence of a percentage mortality in all batches under all the conditions of temperature and moisture content would suggest that internal factors of the organism are to some extent responsible. The accumulation of uric excretions in the tissues, which Roubaud (13) asserts to have an influence upon prepupal duration values may be one such intrinsic factor.

Death in a saturated atmosphere is characterised by a peculiar extension and rigidity of the body, such that the prepupa becomes almost twice the normal length, and by a rapid darkening of the tissues. The full fed larva seems to experience some difficulty in completing the moult which ushers in the prepupal stage.

Under field conditions there is a high degree of mortality among prepupae during periods of continuous infra-zero temperatures and of low moisture conditions. In areas of low rainfall, therefore, the maximum abundance of *Lucilia sericata* may be expected in the vicinity of water courses, and in areas of severe winter temperatures the maximum abundance should occur in urban districts where the prepupae have greater opportunities of securing positions sheltered from frost.

The optimum conditions of soil moisture would seem to be a short range of values on either side of 50 per cent. of saturation, and the migrations of prepupae from the larval medium are carried out with the aim of attaining this "comfort condition" of soil moisture.

Within the temperature range of 10–37° C., and with a soil moisture content around 50 per cent., the duration values of the prepupal stage, expressed in days, are best fitted by a curve of value

$$y = \frac{100}{1{\cdot}17x - 4{\cdot}87},$$

whose reciprocal cuts the temperature axis at 4·2.

VII. The pupal period.

The pupal stage is demarcated somewhat indeterminately from the preceding stage by a loss of mobility and a change in shape from the pyramidal form, of the larva and prepupa, to the characteristic barrel-like form of the muscid puparium.

Once pupation has been attained, the chief significant variable in the environment which influences the duration value is the soil temperature, soil moisture content not appearing to exert an appreciable influence except that extreme dryness may prolong it indefinitely. The early work of Dewitz (4) would suggest that the oxygen content of the soil is an influential factor also, but no data can be offered here in support of this view. Physico-chemical factors of the environment would seem to offer a negligible value of resistance to the potential abundance of this stage. Such resistance as occurs under field conditions is offered by biotic factors.

The following are the duration values for various values of atmospheric temperature. The soil layer was very thin, so that the data are probably in accord with the values obtained under conditions of constant soil temperature. The periods are given in days.

Atmospheric temperature (° C.).

37	30	26	20	17·5	14	10
Never emerged	6–8	7–11	11–13	14–16	18–22	35–45

At temperatures above 30° C., and below 10° C., the duration value is prolonged indefinitely.

The mean values are best fitted by a curve of value

$$y = \frac{100}{0{\cdot}57x - 3{\cdot}2},$$

whose reciprocal cuts the temperature axis at 5·6.

VIII. The total pre-imaginal period.

The duration values for the total pre-imaginal period of the life cycle under conditions of 100 per cent. relative atmospheric humidity, 50 per cent. soil moisture content, and within the range of 10–30° C., are as follows, the values being expressed in days:

Temp.	Egg	Larva	Prepupa	Pupa	Total
30	0·40–0·43	2·0– 3·0	2·5– 4·5	6·0– 8·0	10·9–15·9
26	0·48–0·50	2·5– 3·5	3·0– 6·0	7·0–11·0	13·0–21·0
20	0·81–0·85	4·0– 5·0	3·0– 7·0	11·0–13·0	18·8–25·8
17·5	1·12–1·16	4·5– 5·5	4·0– 8·0	14·0–16·0	23·6–30·6
14	1·98–2·04	7·0– 8·0	6·0–10·0	18·0–22·0	33·0–42·0
10	3·60–3·80	10·0–16·0	11·0–17·0	34·0–46·0	58·6–82·8

The values given for the total period are, however, too low, since the prepupal and pupal stages would be spent normally in soil which is lower in temperature by several degrees than the atmospheric temperature in which the egg and larval stages are spent.

A general correction, however, cannot be applied since it depends upon the values of two factors which are variable, namely:

(i) The depth to which prepupae descend into the soil to pupate,

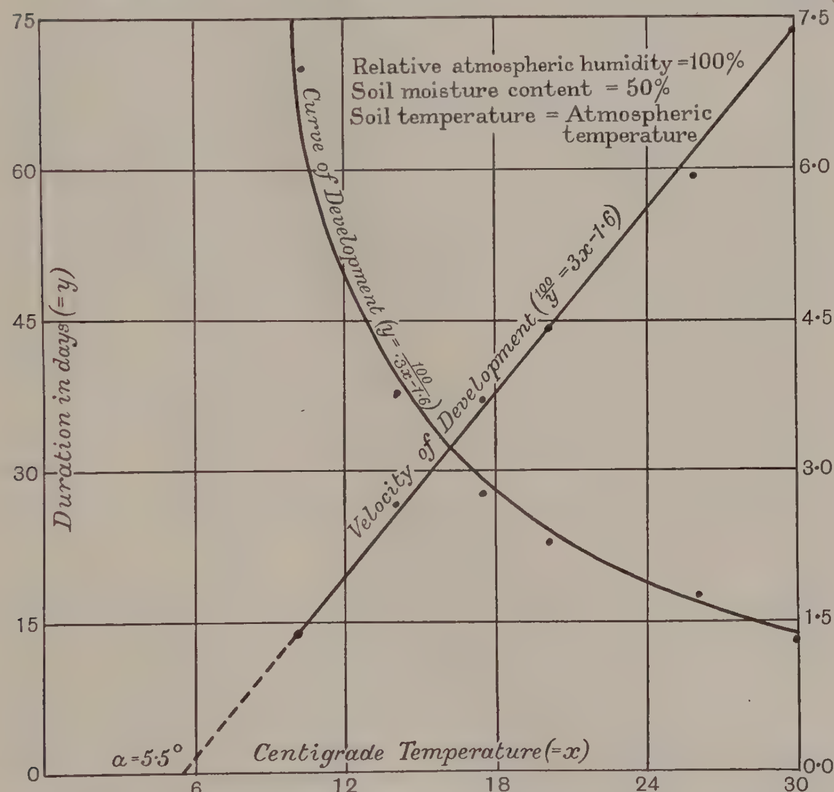

Fig. 1. Curves of development and velocity of development of the pre-imaginal life cycle of *Lucilia sericata* under the conditions given.

a depth which, although not exceeding the water level, and coinciding usually with the level where the soil moisture content is between 40 and 60 per cent., is a factor which will vary in value according to the nature of the soil.

(ii) The difference between atmospheric temperatures and soil temperatures at this depth, which again will vary according to the nature of the soil and according to the nature of the vegetative covering.

General conclusions.

1. The resistance of the environment to the potential abundance of the pre-imaginal life cycle stages of *Lucilia sericata* is most pronounced during the first larval stage and the prepupal stage.

2. The major significant variables in the environment which limit the potential abundance are, in order of importance, as follows:

(i) Values, during the season of imaginal activity, of atmospheric and soil temperatures, outside an optimum range lying between 30° C. and 37° C.; values of relative atmospheric humidity outside an optimum range of 90–100 per cent.; values of protide moisture content outside an optimum of 25–30 per cent.; values of soil moisture content outside an optimum range of 40–60 per cent.; these variables increase the duration values of the life cycle stages, limit the number of annual generations, and limit the potential population density at the commencement of hibernation or aestivation.

(ii) Values, during the season of hibernation or aestivation, of soil temperature outside an optimum range of 10–30° C. and of soil moisture content outside a range of 40–60 per cent. These variables influence the rate of mortality and the duration value of the hibernating or aestivating prepupal stage.

(iii) Unfavourable ratios between the female population density of each generation and the available mass of oviposition media; competition induced between ovipositing females restricts the potential egg production and induces an unfavourable ratio between the number of eggs deposited per unit mass of medium and the number of larvae which can secure feeding positions within the medium.

(iv) Variations in the physico-chemical constitutions of the larval food medium induced by variations of atmospheric humidity, by air movements, by fungi and bacteria. Such changes in the medium curtail the larval stage and induce a high mortality rate among succeeding prepupae and a low proportion of females in the succeeding imaginal phase.

(v) Biotic factors, particularly parasites and predators of the second larval stage.

3. The minor significant variables in the environment include values of relative atmospheric humidity unfavourable to egg incubation; unfavourable values of non-consumable constituent in the larval food medium; air movements, which induce variations in relative humidity, diminish the moisture content of oviposition media and so, by rendering

them unattractive to the ovipositing female and influence the ratio between female population density and available oviposition media; precipitation of atmospheric humidity, which may remove feeding larvae from the medium, may increase the mortality rate among prepupae or may compact the soil surface and so restrict the successful emergence of imagines; predators of the imaginal stage.

4. In an environment in which the temperature and moisture content of the medium, whether air, protide or soil, are within the range of values optimum for the stage under consideration; in which the factor of competition between individuals is minimised by adjustment of the ratio between initial population density and volume of medium; and from which air movement, precipitation, predators and parasites are excluded, the value of environmental resistance to the biotic potential of the stage will equal unity and the final population density will equal the initial population density. They may be in the case of the egg stage a resistance value induced by imperfections of technique, and in the case of the prepupal stage a value of resistance due to intrinsic factors of the organisms themselves.

5. The mean duration values of the total pre-imaginal period of the life cycle at temperatures between 10° C. and 13° C. at a relative atmospheric humidity value of 100 per cent., and a soil moisture content value around 50 per cent., are best fitted by a curve of value

$$y = \frac{100}{0{\cdot}3x - 1{\cdot}6},$$

where y denotes the duration value and x denotes the temperature value. The reciprocal of the curve is a straight line which intersects the temperature axis at 5·5.

The curves for the duration values of the larval, prepupal and pupal stages have reciprocals which cut the temperature axis at 5·3, 4·2 and 5·6 respectively. These values are believed to approximate to the actual threshold temperatures of development and the velocity of development between 10° C. and 30° C. is believed to be uniform for these stages.

In the case of the egg stage, the reciprocal of the curve of duration values, at 14° C. and below, cuts the temperature axis at 5·6, but the reciprocal of the curve of values at temperatures above 14° C. cuts the axis at 9·5. It is suggested that the actual threshold temperature is around 5·6 and that at temperatures above 14° C. there is an acceleration of the velocity of development, correlated with the increasing mass of water vapour per unit of saturated air.

The conclusions given above are based upon data obtained chiefly in the laboratories of the University of Minnesota, and the author acknowledges with gratitude the facilities placed at his disposal by Dr Royal N. Chapman, Chief of the Division of Entomology in the University of Minnesota.

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THE BIOLOGICAL DECOMPOSITION OF PLANT MATERIALS. PART III. PHYSIOLOGICAL STUDIES ON SOME CELLULOSE-DECOMPOSING FUNGI

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(With Plates XXXVII–XXXIX, and 24 Graphs.)

CONTENTS.

I. INTRODUCTION.

When plant materials, such as straw, leaves, etc. are added to soil, or allowed to remain on the ground or in a compost heap, decomposition sets in to a greater or lesser degree. The extent depends upon the conditions obtaining, and the chemical composition of the plant tissues in question. The decomposition is effected by the numerous micro-organisms of all types present. The range and efficiency of the organisms may,

however, vary considerably. In the soil, under normal conditions, saprophytic fungi, aerobic bacteria, and Actinomyces, are the types responsible but, under anaerobic or partially anaerobic conditions as, for example, in waterlogged soil, bacteria alone may be concerned in the process. In such a case the decomposition may follow an entirely different course. Microbiological decomposition is essentially a process of assimilation of the available carbonaceous substance of the tissues. The different structural and reserve materials of plants vary enormously in their availability to particular organisms, whilst the organisms vary similarly among themselves in their ability to attack the substances present. In the assimilation of the various carbon compounds the micro-organisms require a sufficiency of nitrogen in an available form to build up their microbial protein and, since mature plant tissues have, in general, a rather low nitrogen content, the nitrogen supply is not infrequently, in nature, the factor limiting the rate and extent of decomposition.

In the presence of sufficient available nitrogen, decomposition is very rapid. For example, under optimum conditions, Rege (18) observed a a loss of 45 per cent. of the dry matter of rice straw in 6 weeks, 30 per cent. being removed during the first fortnight. In similar work by Norman (16), employing oat straw, a loss of about one-third of the whole was observed in twenty-four days. The most outstanding feature of this decomposition was, however, not so much the very rapid loss of organic matter, as the decomposition of the cellulose over the same period, the loss amounting to 50 per cent. of the total cellulose originally present, equivalent in quantity to two-thirds of the lost organic matter. When active decomposition had ceased, the loss of cellulose amounted to practically 70 per cent. of the total loss of organic matter. The results of analyses carried out on other decomposing plant materials demonstrate similarly that the constituent of the tissues which suffers greatest aggregate loss is the cellulose. The loss of hemicelluloses though greater when calculated as a percentage, is not nearly so great as that of true cellulose. Such a rapid destruction of cellulose implies the presence of organisms very active in the assimilation of carbon in this form. Although cellulose is chemically very stable it is clear that biologically it must be regarded as easily available. It is not necessary to assume that one organism alone is responsible, under natural conditions, for the complete breakdown of the cellulose molecule to its ultimate products, carbon dioxide and water. On the contrary, it is probable that the decomposition often proceeds by stages, being effected by a whole series of organisms each attacking the particular degradation product which most suitably meets its energy

requirements. It is for this reason, perhaps, that our knowledge of the process of cellulose decomposition is in such a disordered condition. On purely constitutional grounds it has been stated that the route of this decomposition is via 4β-glucosido-glucose, or cellobiose, glucose, and the simple organic acids normally arising from sugars. There is, however, apart from the work of Pringsheim (17), little evidence for such an assumption, in fact rather the reverse, for not infrequently it is found that in the decomposition of cellulose by a particular organism there is produced some unusual product. As an example of this may be cited the gum produced by *Spirochaeta cytophaga* (Hutchinson and Clayton (6)) or the production of reducing slimes (van Iterson, jr. (7)). The earlier workers in this field were of the opinion that cellulose decomposition under normal conditions is mainly the work of aerobic bacteria. Many of these have been described and isolated, but in pure culture their ability to decompose cellulose seems frequently to be much reduced, rather supporting the thesis that in nature a secondary microflora plays a not unimportant part. More recently it has been demonstrated that soil fungi are undoubtedly capable of decomposing cellulose, and some workers such as Scales (22), Daszewska (3) and, in particular, Waksman (26), have suggested that they are mainly responsible for the decomposition of cellulosic material in the soil. Actinomycetes have also been shown by Krainsky (11) and Waksman and Curtis (27) to be cellulose decomposers and, since they are common members of the soil microflora, an important place has also been assigned to them by several workers. The addition of natural plant tissues to the soil under normal conditions has been clearly shown greatly to increase the development of fungi. Rege (18) showed that in the presence of available nitrogen certain fungi could together produce a decomposition at least as rapid and complete as that effected by a general soil microflora, while bacteria alone were very considerably slower. To a certain extent this was perhaps due to the conditions of the experiment, but the very rapid early loss of organic matter that occurred both with fungi alone and with a general microflora, strongly suggests the important rôle which the fungi may play. Further evidence on this point will be given in a later communication.

SCHEME OF WORK.

A technique for the evaluation of the more important plant constituents having been evolved (Norman (15)), it was possible to follow in detail the losses in each group sustained by straws rotting by the agency of a natural microflora. An account of this work has appeared elsewhere

(Norman (16)). It was then thought desirable to follow the particular biochemical activities on natural materials of certain of the individual organisms isolated in the preceding experiments, with the intention of throwing more light on the rôle of the fungi in these processes. In this paper will be described certain physiological characteristics and biochemical reactions on synthetic media of a number of fungi isolated from straws undergoing active decomposition, while in a later paper will be outlined their specific biochemical activities on the natural straws.

II. EXPERIMENTAL.

Isolation of organisms.

Samples of oat and rye straws were bottled, and available nitrogen added as ammonium nitrate to the extent of 1 gm. nitrogen to 100 gm. dry straw; water was added from a spray till the straws were thoroughly wet, but not waterlogged. An inoculum from cultivated soil was added to each. Since in manure and compost heaps the temperature often rises as high as 50° C., certain bottles were incubated at that temperature, so that the growth of organisms active under such conditions might be assisted. Other bottles were incubated at 30° C. Chemical analyses have shown that, under these conditions, decomposition is most rapid or, in other words, the organisms are most active, from about the fourth to the twelfth day. Accordingly, at the end of seven days, platings at a dilution of 1 in 50,000 on the wet straw were made on the following media: Conn's agar (pH 4·6); Czapek's agar (pH 7·6), Waksman's agar (pH 4·0); cellulose agar (pH 4·5); cellulose agar (pH 7·6). The cellulose agar was prepared according to the formula of McBeth and Scales (12) and contained 5–6 gm. re-precipitated cellulose per litre. After incubation at 30° C. and 50° C. respectively, according to the source, there was growth on practically all plates. Bacterial colonies were numerous on the Czapek medium. Growth on the cellulose plates was in all cases scanty and would not have led to the conclusion that there were present any active cellulose decomposing organisms. However, evidence will be presented later to show that practically all the forms isolated utilise readily the natural cellulose of straw.

Inability, therefore, to develop on a cellulose-agar plate cannot be taken as evidence that the organism in question is not naturally a cellulose decomposer. It would seem that much of the apparent obscurity of the whole process of cellulose decomposition may not improbably be ascribed to this cause.

Purification was effected by sub-culturing from acid plates to Czapek's agar and back, and from Czapek's agar and neutral cellulose plates on to Conn's agar. Some difficulty was experienced with those at 50° C., as will be explained later.

The following organisms were isolated:

50° C.	30° C.
Sepedonium sp. (Acremoniella)	*Aspergillus fumigatus*
Aspergillus fumigatus	*Aspergillus niger*
Actinomyces α	*Aspergillus* sp. (*nidulans*)
	Aspergillus sp. (*terreus*)
	Trichoderma sp.
	Actinomyces β

The above-mentioned fungi do not, of course, represent all the organisms that might be present, but it is a noteworthy fact that *Coprinus* sp. (*fimetarius*?), which was frequently isolated by Rege (18) and constantly present, only occurred in one bottle of fermenting straw during the present investigation.

NOTES ON THE IDENTIFICATION OF THE ORGANISMS.

Sepedonium sp. (probably *S. lanuginosus* Miehe).

This is undoubtedly similar to the organism isolated by Rege (18) from rotting straw and described by him as *Acremoniella* sp. (probably *A. velutina* Fuck). It also agrees fairly closely with the description by Tsilinsky (25) of an organism which she named *Thermomyces lanuginosus*. She did not, however, give any dimensions, and the optimum temperature given by her is rather higher than that of the organism obtained in this work. Miehe (13) isolated a similar organism from heating hay and reported it under the name of *T. lanuginosus* (Tsilk). Griffon and Maublanc (4), in their work on thermophilic organisms, preferred the name of *Sepedonium lanuginosum* (Miehe). Recently, through the courtesy of the Imperial Bureau of Mycology, the author received from Prof. M. Curzi of Rome a culture morphologically similar to that isolated in the course of this work. Comparisons of the two carried out on a number of different media revealed only minor cultural differences, and there can be little doubt but that Curzi's organism is a closely related strain. He has given to it the name of *Acremoniella thermophila*. It would seem, however, that the name given by Miehe (13), namely, *Sepedonium lanuginosus*, should be preferred. This organism shows a peculiar tendency to grow on

and over the colonies of other organisms on a mixed plate, rendering separation a matter of some difficulty.

Actinomycetes.

No attempt has been made to identify the two Actinomycetes further. The one described as *Actinomyces* α isolated from straw rotting at 50° C. was a definite thermophilic form, distinct from *Actinomyces* β which was unable to grow at 50° C.

Aspergillus fumigatus.

At 50° C. the growth of this organism is, morphologically, rather unlike the growth at lower temperatures. The hyphae are hyaline, much branched and larger, but sterile. When allowed to remain at room temperature, after growth at 50° C., spores are slowly formed, but are of a light blue-green colour. This strain produced no perithecia at any temperature.

Aspergillus nidulans Eid.

The appearance and measurements of the strain isolated agree with those cited by Thom and Church (23). The perithecia are numerous, and of the order of 300μ in size, with purple walls and polygonal Hülle cells. The ascospores are smooth, pink to purple in colour, oval in shape, and approximately $5 \times 3{\cdot}5\mu$ in size. They have a marked equatorial band round the longer axis which splits on germination.

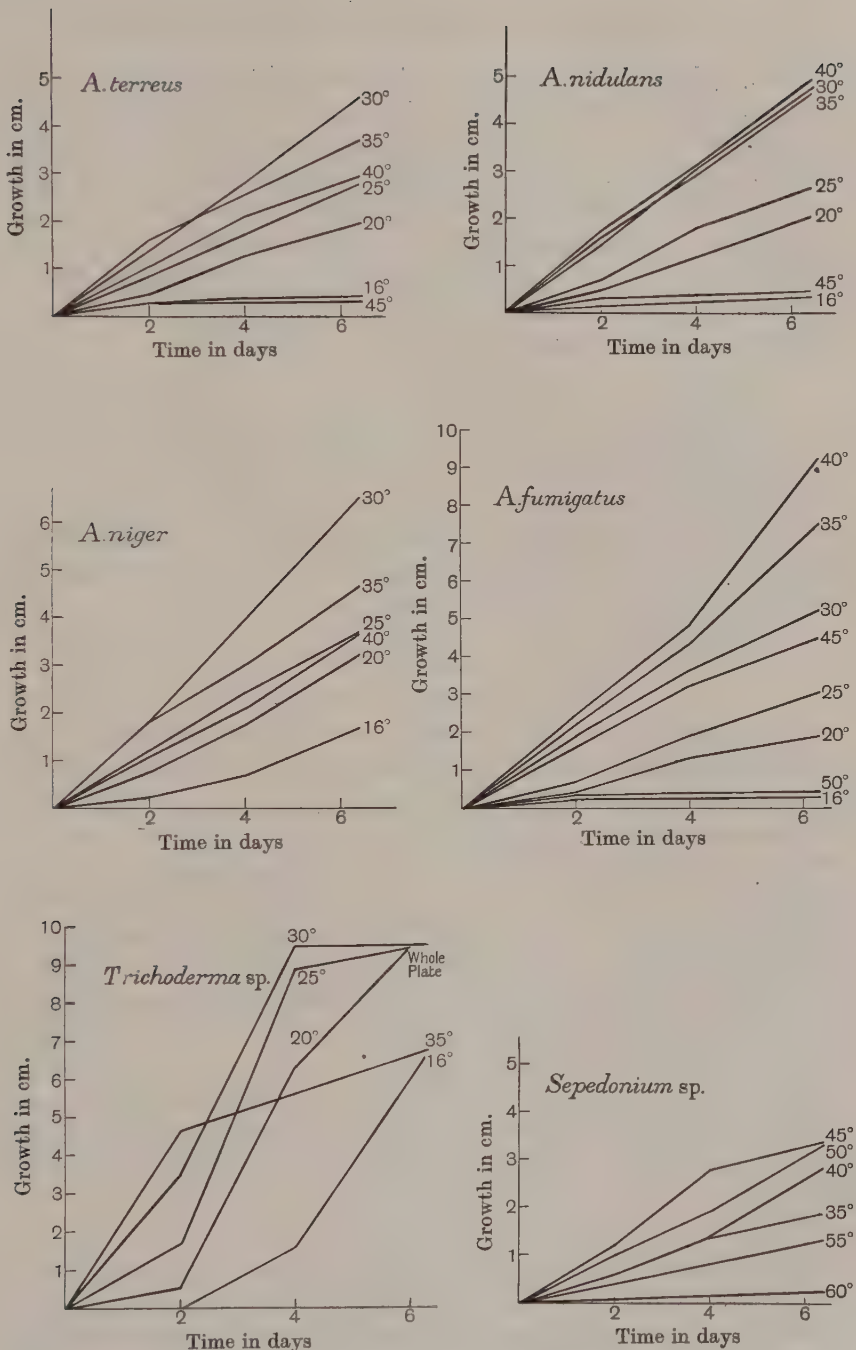
Aspergillus terreus Thom.

The organism obtained is undoubtedly similar to that described by Thom and Church (23). The anastomosing chains of conidia form solid columnar masses which may be 500–600μ in length. The individual conidia are rather over 2μ in diameter. No perithecia are found. The growth and measurements agreed with a culture from the Imperial Bureau of Mycology, isolated originally from paddy soil in Burmah.

III. PHYSIOLOGICAL STUDIES.

(1) Temperature relationships.

Since the temperature of rotting straw frequently rises considerably, it was thought to be of interest to determine the optimum and maximum temperatures of growth of these organisms. This was carried out on Waksman's agar (pH 4·0), which is a rich medium, glucose being the carbohydrate source and peptone that of nitrogen. Six Petri dishes, containing equal amounts of medium, were employed for each temperature,



Graphs I–VI. Growth of fungi at various temperatures.

and inoculations made from a suspension of spores in sterile water. Those at 40° C. and upwards were placed in special containers holding water to avoid drying of the medium. Measurements of the growth of the colonies were taken along two diameters at right angles every two days. It was, of course, not possible to treat the Actinomyces in the same way, since their growth is extremely slow, and the colonies produced are not circular or regular. The results obtained are shown in Graphs I–VI and in Plates XXXVII and XXXVIII. The optimum temperatures on Waksman's medium are:

	° C.		° C.
Trichoderma sp.	30	*Aspergillus nidulans*	40
Aspergillus terreus	30	*Aspergillus fumigatus*	40
Aspergillus niger	30	*Sepedonium* sp.	45

It will be seen that these fungi on synthetic media have their optima, and are active at temperatures above those usual for fungal growth. Some evidence will be given later to show that, under natural conditions, the temperature range may even be extended.

(2) Availability of various carbonaceous and nitrogenous substances.

The growth of these fungi on media containing different carbon and nitrogen compounds was carried out as described above in the temperature relationship studies. The plates were in each case incubated at the optimum temperature for the growth of the fungus concerned. Measurements of colony diameter were taken at the end of seven days. This, however, was not found to be entirely satisfactory for comparative purposes, owing to the variations in type of growth. To meet this, a purely arbitrary factor was devised, based on sporulation and growth, and determined by inspection only. Under this system + 6 represents the maximum observed degree of growth and sporulation, and + 5, + 4, ... + 1 decreasing stages.

The media employed contained 2 gm. of the source of nitrogen, and 15 gm. of the carbon compound per litre of the following mineral salt solution: $MgSO_4$ 0·5 gm.; KH_2SO_4 1·0 gm.; KCl 0·5 gm.; $FeSO_4$ trace; Agar 16 gm.

Nitrogen compounds.

The nitrogenous substances first received attention. One each of the various classes was selected, and sucrose employed as the source of carbon in each case. The results are tabulated below. The colours are referred to Ridgway's *Color Standards and Color Nomenclature* (19).

Growth of organisms on various nitrogenous compounds.

Table I.

Aspergillus terreus.

Source of carbon: Sucrose, 7 days' growth at 30° C.

Source of nitrogen	Mean diameter of colonies in cm.	Growth and sporulation	Colour of colony	Colour in medium
Sodium nitrate	2·0	+2	Cinnamon. 15″ Y–O (XXIX)	Lemon yellow. 23 Y (IV)
Ammonium carbonate	2·0	+3	Sayal brown. 15″ Y–O. i (XXIX)	Lemon chrome to aniline yellow. 21 O–YY (IV) to 19 YO–Y. i (IV)
Peptone	5·0	+3	Cinnamon. 15″ Y–O (XXIX)	Lemon yellow. 23 Y (IV)
Asparagine	2·7	+2	Snuff brown. 15″ Y–O. k (XXIX)	Pale green yellow. 27 G–Y. f (V)
Casein (3 gm. per litre)	4·0	+1	Pinkish cinnamon. 15″ Y–O. b (XXIX)	Martius yellow. 23 Y. f (IV)

Table II.

Aspergillus nidulans.

Source of carbon: Sucrose, 7 days' growth at 40° C.

Source of nitrogen	Mean diameter of colonies in cm.	Growth and sporulation	Colour of colony	Colour of reverse
Sodium nitrate	5·5	+4	Jade green. 27″ G–Y. k (XXXI)	Faint brown
Ammonium carbonate	3·3	+2	Centre, ivy green; outer, Danube green. 25″ YG–Y. m (XXXI). 35″ G. m (XXXII)	Centre wrinkled, brownish olive; outer, yellowish olive. 19″ YO–Y. m (XXX). 23″ Y. k (XXX)
Peptone	4·9	+4	Dark cress green. 29″ GG–Y. m (XXXI)	Slight olive lake. 21′ O–YY. i (XVI)
Asparagine	4·5	+4	Light to dark cress green. 29″ GG–Y. k to m (XXXI). Many sandy perithecia	Raw sienna to olive lake. 17 O–Y. i (III) to 21′ O–YY. i (XVI)
Casein (3 gm. per litre)	6·0	+1	Much white vegetative growth	None

Table III.

Aspergillus fumigatus.

Source of carbon: Sucrose, 7 days' growth at 40° C.

Source of nitrogen	Mean diameter of colonies in cm.	Growth and sporulation	Colour of colony	Colour of reverse
Sodium nitrate	6·0	+5	Dark greyish olive. 21'''' O–YY. k (XVLI)	None
Ammonium carbonate	4·5	+4	Slate olive to deep slate olive. 29'''' GG–Y. k–i (XLVII)	None
Peptone	5·0	+3	Elm green to dusky green. 27' G–Y. m (XVII) to 37'' GB–G. m (XXXIII)	None
Asparagine	8·0	+5	Centre, deep slate green; outer, Russian green. 33''''GY–G. k (XLVII). 37''' GB–G. i (XLII)	None
Casein (3 gm. per litre)	Whole plate	+1	Few scattered spores, slate green. 33'''' GY–G. k (XLVII)	None

Table IV.

Aspergillus niger.

Source of carbon: Sucrose, 7 days' growth at 30° C.

Source of nitrogen	Mean diameter of colonies in cm.	Growth and sporulation	Colony	Colour of reverse
Sodium nitrate	5·0	+5	Large black conidial heads	None
Ammonium carbonate	4·0	+5	Large black conidial heads	None: slightly wrinkled
Peptone	4·6	+5	Large black conidial heads	None
Asparagine	5·0	+4	Large black conidial heads	None
Casein (3 gm. per litre)	5·0	+2	Medium: black conidial heads, white vegetative growth	None

Table V.

Trichoderma sp.

Source of carbon: Sucrose, 7 days' growth at 30° C.

Source of nitrogen	Mean diameter of colonies in cm.	Growth and sporulation	Colour of colony	Colour of reverse
Sodium nitrate	Whole plate	+1	Feeble scattered growth, light bice green. 29″ GG–Y. i (XVII)	None
Ammonium carbonate	,,	+2	Close at centre, scattered otherwise. Dark cress green. 29″ GG–Y. m (XXXI)	None
Peptone	,,	+4	Grouped spores. Pois green. 29‴ GG–Y. i (XLI)	None
Asparagine	,,	+3	Grouped spores, centre glass green. 29″ GG–Y. d (XXXI). Edge, cream buff. 19″ YO–Y. d (XXX)	None
Casein (3 gm. per litre)	,,	+3	Grouped spores: deep dull yellow green. 31″ Y–G. k (XXXII)	None

Table VI.

Sepedonium (*Acremoniella*).

Source of carbon: Sucrose, 7 days' growth at 50° C.

Source of nitrogen	Mean diameter of colonies in cm.	Growth and sporulation	Colour of colony	Colour of reverse
Sodium nitrate	7·5	+1	Spreading white vegetative growth, slimy	None
Ammonium carbonate	Germinates only	—	—	—
Peptone	4·8	+2	Moist white growth, faint courge green at centre. 25′ YG–Y. i (XVII)	None
Asparagine	8·0	+6	Submerged margin, light drab. 17⁗ O–Y. b (XLVI). Centre, deep greyish olive. 21⁗ O–YY. i (XLVI) to fuscous, 13⁗ OY–O. k (XLVI). Sectoring observed	Tawny olive. 17″ O–Y. i (XXIX)
Casein (3 gm. per litre)	7·8	+2	Moist white growth: spores in centre, deep olive. 21‴ O–YY. k (XL)	None

It will be seen that casein, which was selected as a representative protein, is utilised, to any extent, only by *Trichoderma* sp. In the other cases not only was growth affected, but also the production of colour in the spores. Asparagine is especially suitable for *Sepedonium* sp. which gives on it a luxuriant growth. Rege (18) reported asparagine as being much less suitable than peptone for this organism. It would seem likely therefore that a different physiological strain of this fungus was isolated by him. The Aspergilli with the exception of *Aspergillus terreus* also develop well upon it. Both nitrate and ammonia are suitable for the Aspergilli. *Trichoderma* sp. and *Sepedonium* sp., however, grow better on an organic source of nitrogen. *Sepedonium* sp. gives but a scanty growth with nitrate and only germinates with ammonia as the source of nitrogen. Of the substances tried, peptone is the most generally utilisable form, and for this reason was employed as the source of nitrogen in the studies in which the carbonaceous source was varied. It was not wholly satisfactory for *Sepedonium* sp. in the presence of sucrose, but there is evidence that this organism reacts more to change of carbonaceous material than to nitrogen, provided that the latter is supplied in an organic form.

Carbon compounds.

In investigating the availability of various carbohydrate compounds a representative selection of hexoses, pentoses, disaccharides and polysaccharides was made. Particular attention was given to those sugars known to form units of the hemicellulose molecule in straws. These are arabinose, xylose and galactose. Gum arabic was also included since it resembles in units and constitution the structural hemicelluloses. Pectin, being a conjugated derivative of the same type, containing arabinose, galactose and tetra-galacturonic acid and occurring in most plant materials other than woods, was also tested both alone and in the presence of chalk. The results are summarised in Tables VII–XII, an arbitrary factor for growth being employed as in the case of the nitrogenous substances.

A survey, from the point of view of the constitution of the various carbohydrates concerned, shows several interesting features revealed only by the employment of the arbitrary factor for growth and sporulation for, in many of the cases in which the growth is thin and the sporulation scanty, the mean size of the colony is above average. Numerical comparison is only truly valid between cultures of the same organism and not between different organisms.

Glucose is, in all cases as good as and, in some cases, particularly that of *A. fumigatus*, distinctly better than sucrose as the carbohydrate source. Invertase production in *A. fumigatus* must, therefore, be slow.

The four Aspergilli grow better on glucose which is an aldohexose than on fructose which is a ketose, but this does not apply to *Trichoderma* sp. or *Sepedonium* sp., both of which give a heavy growth on a fructose medium.

Growth of organisms on various carbon compounds.

Table VII.

Aspergillus terreus.

Source of nitrogen: Peptone, 7 days' growth at 30° C.

Source of carbon	Mean diameter of colonies in cm.	Growth and sporulation	Colour of colony	Colour in medium
Sucrose	5·0	+3	Cinnamon. 15″ Y–O (XXIX)	Lemon yellow. 23 Y (IV)
Glucose	4·8	+4	,,	Pyrite yellow to greenish yellow. 23 Y. i (IV) to 25 YG–Y (V)
Maltose	3·6	+4	,,	Strontian yellow. 23′ Y (XVI)
Xylose	4·0	+3	,,	Strontian yellow. 23′ Y (XVI)
Arabinose	3·8	+3	,,	Reed yellow. 23″ Y. b (XXX)
Galactose	3·5	+4	,,	Aniline yellow to lemon chrome. 19 YO–Y. i (IV) to 21 O–YY (IV)
Lactose	3·0	+1	,,	Barium yellow. 23′ Y. d (XVI)
Fructose	3·0	+2	,,	Strontian yellow. 23′ Y (XVI)
Starch	3·5	+2	,,	Pale lemon yellow to Martius yellow. 23 Y. b (IV) to 23 Y. f (IV)
Gum arabic	3·3	+1	White to light pinkish cinnamon. 15″ Y–O. d (XXIX)	None
Pectin	6·5	+4	Cinnamon. 15″ Y–O (XXIX)	Wax yellow. 21′ O–YY (XVI)
Pectin and chalk	1·3	+1	Cinnamon buff. 17″ O–Y. b (XXIX)	Slight

Galactose, the other hexose tested, is frequently found in hemicelluloses. It is as satisfactory or better than glucose for three Aspergilli and *Trichoderma* sp., slightly less so for *A. fumigatus*, and only unsatisfactory for *Sepedonium* sp. which grows well but fails to sporulate on it.

Of the disaccharides, maltose is only as suitable as glucose for *Sepedonium* sp. and *A. terreus*, being apparently rather less available to the

Growth of organisms on various carbon compounds.

Table VIII.

Aspergillus nidulans.

Source of nitrogen: Peptone, 7 days' growth at 40° C.

Source of carbon	Mean diameter of colonies in cm.	Growth and sporulation	Colour of colony	Colour of reverse
Sucrose	4·1	+4	Close growth, dark cress green. 29″ GG–Y. m (XXXI)	Olive in centre. 21″ O–YY. m (XXX)
Glucose	5·5	+4	Close growth, cress green. 29″ GG–Y. m to k (XXXI)	None
Maltose	5·5	+3	Grass green. 33 GY–G. k (VI)	None
Xylose	6·0	+3	Cedar green. 31 Y–G. m (VI)	None
Arabinose	3·8	+3	Outer, white; centre, Pois green. 29‴ GG–Y. i (XLI)	None
Galactose	6·0	+5	Close growth, civette green. 31′ Y–G. k to m (XVIII)	None
Lactose	4·0	+2	Spores grouped, jade green. 27″ G–Y. k (XXXI)	Lime green. 25″ YG–Y (XXXI)
Fructose	4·5	+3	Elm green. 27′ G–Y m (XVII)	Light yellowish olive. 23″ Y. i (XXX)
Starch	3·5	+3	Loose growth, elm green. 27′ G–Y. m (XVII)	Greyish olive and cinnamon buff. 21⁗ O–YY (XLVI) to 17″ O–Y. b (XXIX)
Gum arabic	5·0	+1	White growth: centre, light yellowish olive. 23″ Y. i (XXX)	None
Pectin	5·0	+ 3	Roman green. 23′ Y. m (XVI)	None
Pectin and chalk	3·0	+1	Scanty growth, Kronberg's green. 25″ YG–Y. k (XXXI). Small sandy perithecia	None

others tested. Since maltose in an α-glucoside, it would seem to indicate the slow production of an α-enzyme in these cases.

Although both glucose and galactose are utilised readily, lactose, which is a β-glucoside of the latter, is only readily available to *Trichoderma* sp., which must therefore contain a β-enzyme.

Growth of organisms on various carbon compounds.

Table IX.

Aspergillus fumigatus.

Source of nitrogen: Peptone, 7 days' growth at 40° C.

Source of carbon	Mean diameter of colonies in cm.	Growth and sporulation	Colour of colony	Colour of reverse
Sucrose	5·0	+3	Elm green to dusky green. 27′ G–Y. m (XVII) to 37″ GB–G. m (XXXIII)	None
Glucose	7·5	+6	Slate olive. 29″″ GG–Y. i (XLVII)	None
Maltose	8·5	+3	Slate olive. 29″″ GG–Y. k (XLVII)	None
Xylose	5·5	+2	Scanty growth, Andover green. 25″″ YG–Y. i (XLVII)	None
Arabinose	7·5	+4	Slate olive. 29″″ GG–Y. k–i (XLVII)	None
Galactose	6·6	+4	Dark ivy green. 25″″ YG–Y. k (XLVII)	None
Lactose	7·0	+2	Scanty growth, dark ivy green. 25″″ YG–Y. k (XLVII)	None
Fructose	8·0	+3	Dark ivy green. 25″″ YG–Y. k (XLVII)	None
Starch	8·2	+3	Sage green to slate olive. 29″″ GG–Y (XLVII) to 29″″ GG–Y. i (XLVII)	None
Gum arabic	8·8	+1	Deep grape green. 25‴ YG–Y. i (XLI). Zonation observed	None
Pectin	5·5	+4	Dark greenish olive. 23″ Y. m (XXX)	None
Pectin and chalk	Whole plate	+2	Dark ivy green. 25″″ YG–Y. k (XLVII)	None

Of the polysaccharides, starch is as available as its structural unit, maltose, to all organisms tested, with the exception of *A. niger* and *A. terreus*, in which amylase production apparently lags behind that of maltase.

As mentioned previously, particular interest attaches to the pentose sugars owing to their presence in hemicelluloses. The two tested are the only two commonly found in plant materials, namely, xylose and arabinose. The former is believed to arise in nature by the degradation of glucose and the latter similarly from arabinose. Both the pentose sugars are available to all the organisms tested, but are not quite so suitable as their sterically related hexoses except to *Sepedonium* sp. which develops

Growth of organisms on various carbon compounds.

Table X.

Aspergillus niger.

Source of nitrogen: Peptone, 7 days' growth at 30° C.

Source of carbon	Mean diameter of colonies in cm.	Growth and sporulation	Colony	Reverse
Sucrose	4·6	+5	Large black conidial heads, white vegetative growth	No colour
Glucose	5·0	+5	,,	,,
Maltose	5·8	+4	,,	,,
Xylose	7·0	+4	Smaller black conidial heads	,,
Arabinose	5·5	+3	Scattered conidial heads, medium size	,,
Galactose	7·7	+6	Large black heads	,,
Lactose	4·7	+2	Thin growth, scattered but large conidial heads	,,
Fructose	4·2	+4	Large black heads	,,
Starch	4·2	+2	Smaller black heads	,,
Gum arabic	5·5	+1	Few large heads	,,
Pectin	7·0	+5	Large black conidial heads	,,
Pectin and chalk	5·0	+4	Large conidial heads. Chalk markedly dissolved out in broad zone round colony	,,

better on the pentose sugar. This is in direct contradiction to the observations made on this fungus by Rege (18), who found that for his strain the pentoses were poor nutrients. Commercial preparations of both these sugars frequently contain copper to a not inconsiderable extent, and this would, of course, have the effect of inhibiting growth. Rege's organism gave good growth on a mixed hemicellulose preparation from straw, which contained these two sugars, rather supporting the view that some

inhibitory substance was present in the pentose sugars employed by him. Of the two pentoses, xylose is more suitable than arabinose for *Sepe-*

Growth of organisms on various carbon compounds.

Table XI.

Trichoderma sp.

Source of nitrogen: Peptone, 7 days' growth at 30° C.

Source of carbon	Mean diameter of colonies in cm.	Growth and sporulation	Colour of colony	Reverse
Sucrose	Whole plate	+4	Grouped spores, pois green. 29‴ GG–Y. i (XLI)	No colour
Glucose	,,	+4	Grouped spores, deep dull yellow green. 31″ Y–G. k (XXXII)	Medium clouded
Maltose	,,	+2	Very scattered clumps of spores, dark cress green. 29″ GG–Y. m (XXXI)	No colour
Xylose	,,	+4	Scattered groups, deep dull yellow green. 31″ Y–G. k (XXXII)	Cloudy deep buff. 21″ O–YY. b (XXX)
Arabinose	,,	+4	Grouped spores, dark cress green. 29″ GG–Y. k to m (XXXI)	No colour
Galactose	,,	+5	Grouped spores, dusky yellowish green. 29‴ GG–Y. m (XLI)	No colour
Lactose	,,	+5	Grouped spores, dark American green. 33‴ GY–G. k (XLI)	No colour
Fructose	,,	+6	Closely grouped spores, leaf green. 29‴ GG–Y. k (XLI)	Grape green. 25‴ YG–Y (XLI)
Starch	,,	+4	Grouped spores, dull blackish green. 33‴ GY–G. m (XLI)	Clear dull green yellow. 27′ G–Y. b (XVII)
Gum arabic	,,	+2	Grouped spores, dark cress green. 29″ GG–Y. m (XXXI)	No colour
Pectin	,,	+1	Grouped spores, civette green. 31′ Y–G. k (XVIII)	No colour
Pectin and chalk	,,	+3	Fluffy loose growth, civette green. 31′ Y–G. k (XVIII)	No colour

donium sp. and less so for *A. fumigatus*. The remaining organisms appear to develop equally well on either.

Growth of organisms on various carbon compounds.

Table XII.

Sepedonium (Acremoniella).

Source of nitrogen: Peptone, 7 days' growth at 50° C.

Source of carbon	Mean diameter of colonies in cm.	Growth and sporulation	Colour of colony	Colour of reverse
Sucrose	4·8	+2	Moist white growth, faint courge green at centre. 25′ YG–Y. i (XVII)	None
Glucose	5·0	+3	White vegetative growth, spores deep grape green. 25‴ YG–Y. i (XLI)	Faint leaf green. 29‴ GG–Y. k (XLI)
Maltose	7·0	+4	Slimy smooth vegetative growth: outer spores, Kronberg's green. 25″ YG–Y. k (XXXI); inner, Lincoln green. 25‴ YG–Y. k (XLI)	Yew green. 27″ G–Y. m (XXXI)
Xylose	6·5	+5	Slimy vegetative growth, spores dark ivy green. 25⁗ YG–Y. k (XLVII). Sectoring observed	Dusky olive green. 25‴ YG–Y. m (XLI)
Arabinose	4·8	+3	Spores dark olive grey. 23′′′′′ Y. i (LI)	Faint brown
Galactose	6·0	+1	Good white vegetative growth. No spores produced	None
Lactose	7·5	+1	Spreading vegetative growth. No spores	None
Fructose	6·5	+5	White vegetative growth, dark ivy green spores. 25⁗ YG–Y. k (XLVII). Sectoring observed	Dark ivy green. 25⁗ YG–Y. k (XLVII)
Starch	7·2	+4	White vegetative growth. Spores, dark ivy green. 25⁗ YG–Y. k (XLVII) to olivaceous black. 25⁗ YG–Y. m (XLVII)	Olivaceous black. 25⁗ YG–Y. m (XLVII)
Gum arabic	7·2	+2	White vegetative growth, spores deep greyish olive. 21⁗ O–YY. i (XLVI)	None
Pectin	3·5	+1	White vegetative growth	None
Pectin and chalk	4·0	+2	Olive brown. 17‴ O–Y. k (XL)	None

Gum arabic, which consists of arabinose linked through a glucoside linkage to a nucleus acid containing galactose and galacturonic acid is, rather unexpectedly, not very suitable for any of the organisms. All make extensive spreading growth, but produce few spores, the Aspergilli forming even less than *Trichoderma* sp. and *Sepedonium* sp.

Pectin, which is another substance of this type, is utilised readily by the Aspergilli, particularly by *A. niger*, but hardly at all by *Trichoderma* sp. and *Sepedonium* sp. During the decomposition the medium becomes very acid. Another series was set up in which 20 gm. of precipitated chalk per litre was added. Under these circumstances, in which the medium is kept neutral, the position is reversed, since the Aspergilli, with the exception of *A. niger*, give but scanty growth, while *Trichoderma* sp. and *Sepedonium* sp. grow rather more readily than before. Norman (16) has shown that the small amount of pectin present in straw is not removed early in the process of decomposition under normal aerobic conditions, but remains till a late stage. If, however, the reaction of the rotting material becomes acid, which is an abnormal condition, the pectin rapidly disappears. It is clear that these two observations are closely related.

Variation on certain media.

The general habit of growth of the Aspergilli in question and also of *Trichoderma* sp. does not seem to be markedly affected by changes in the carbohydrate source. The colour of the spores, degree of sporulation and the colour in the medium may be altered somewhat, but the colonies of the same organism on different media bear a general resemblance one to another, and are easily recognisable. The same may not be said of *Sepedonium* sp. which exhibits widely different habits of growth on different media. So diverse are the forms and colours produced that the colony is of little assistance in identification. Furthermore, as noted by Rege (18), cultures of this organism on several media, and particularly on certain carbohydrates, exhibit the phenomenon known as "sectoring," in which a wedge of a discontinuous variant bearing light-coloured spores appears, as shown in Plate XXXIX. Sectoring was not infrequent on xylose, and fructose, with peptone as the source of nitrogen. It was observed once only in a large number of cultures upon asparagine with sucrose as the source of carbon. Sub-cultures from the light and the dark or normal portions have remained constant through six or seven transfers, and have never exhibited further sectoring or variations. In no case has the light sporing form reverted. The spore size is identical in the two

forms but the habit of growth and the colour produced in various media are different. Generally speaking, the light sporing form has a looser growth, and the spores are not clumped quite so definitely as in the normal form. "Sectoring" or any other manifestation of variation was not observed in any of the other fungi investigated.

(3) Thermogenesis.

That the natural decomposition of plant materials in a heap or stack is often accompanied by an evolution of heat and a rise in temperature of the heap is a matter of common knowledge. Particularly is this observed in the case of fresh or partly dried materials such as hay or green fodder during ensilage. It occurs also if dried materials such as cereal straws or tobacco leaves are moistened and allowed to rot. Farmyard manure, similarly, shows a rise in temperature during the "ripening" process.

Three theories have been put forward to account for this phenomenon in all but the last example. Many investigators have ascribed it to microbiological activity, and Miehe (13) studied the production of heat in partly sterilised hay inoculated with various organisms. Others, such as Russell (20) in the case of silage, and Tschirch (24) for hay, suggested that the heating is, in the main, due to the action of plant enzymes. Boekhout and de Vries (1) have put forward a chemical explanation for the heating of hay and postulate an oxidative process assisted by inorganic catalysts, such as iron, in the tissues. More recent work has led to the view that each of these actions may take place, but to a very different degree in various cases. Jones and Gibbard (10) have shown in the case of ensilage that, although plant enzymes may be active during the early stages, the micro-organisms present play the chief part in the changes observed. Similarly, Haldane and Makgill (5), investigating the spontaneous combustion of hay, came to the conclusion that simple chemical oxidation is concerned in addition to the action of micro-organisms. The oxidative process is, however, not appreciable until the temperature has been raised very considerably by the activity of the organisms. They agree with Miehe (14) that one group of organisms raises the temperature to about 40° C. first, and thermophilic organisms increase this to 60–70° C. Any further increase is due to chemical oxidation alone, since the hay is found to be sterile above this point. In all these processes it would seem that the activity of micro-organisms plays an important, though not necessarily exclusive, part in the raising of the temperature. Despite these observations, there have been but few attempts to determine the

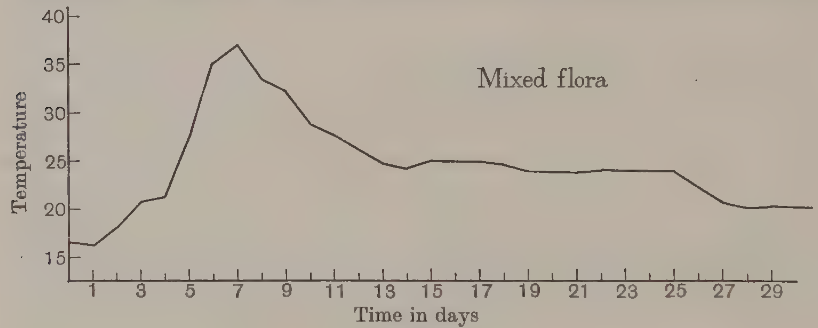
thermogenic powers of individual micro-organisms. Since the organisms isolated in the course of this work develop actively on synthetic media at temperatures above those usual for fungal growth, it appeared to be of interest to determine whether single organisms upon natural materials could themselves bring about any appreciable rise of temperature.

James (8) has recently described an apparatus which may be employed for measuring the temperature developed in an organic material after inoculation. It consists of a glass container which, with its contents, may be sterilised and placed inside a Dewar flask, the whole being surrounded with insulating material. The rotting material may be aerated at will by displacement from an aspirator. He showed that heating does not take place in the total absence of air or oxygen. This apparatus has been used by James, Rettger and Thom (9) in studies on the heat production in cornmeal. During the decomposition, however, they passed through the flask a steady stream of oxygen. The conditions inside the flask can, therefore, hardly be regarded as normal, since there is a constant supply of oxygen and a removal of carbon dioxide. The oxygenation is of great significance for, to quote one example only, oats when aerated with oxygen showed a temperature of 49·2° C., but when unaerated only 39·1° C. In the present work it was thought that the conditions of decomposition should adhere as closely as possible to natural conditions, and that aeration with oxygen is irreconcilable with this object. The temperatures that are here recorded are, therefore, those likely to be obtained naturally, or rather lower, since the actual bulk of material taken was in each case small and heat losses through radiation proportionately larger. James, Rettger and Thom (9) stated that, for cornmeal, the maximum temperatures produced appeared to be affected by the moisture content of the material only within rather wide limits. Nevertheless, it was considered desirable in the work to be described that the initial moisture content should be kept uniform throughout.

Unsterilised straw.

Since the organisms described herein were all isolated from decomposing straw, chaffed oat straw was employed as the substrate in these investigations. It was of importance first to determine the increase of temperature brought about on unsterilised straw by the mixed flora which it carries. 45 gm. of oat straw, chaffed small, was placed in a Dewar vacuum flask and wetted by the addition of 100 c.c. of water containing 1·4 gm. ammonium nitrate. The flask was plugged with wool through which ran a thermometer, and placed on its side, being frequently turned

in the earlier stages to facilitate thorough wetting. The flask temperature was read daily at 9 a.m. and the room temperature also recorded. This is of importance, because it was noted that fluctuations in room temperature were reflected in the temperature of the straw, since the vacuum flasks employed were not very efficient in the retention of heat. The heat losses observed were approximately 1° C. per hour from 50° C., 0·6° C. per hour from 40° C., 0·35° C. per hour from 30° C., and 0·1° C. per hour from 20° C. at a room temperature of 16° C. In order that the effect of fluctuations in air temperature might be avoided and separate series at various times made comparable, the difference between the room temperature and that of the flasks on any day is added to the mean figure for air temperature readings over the whole period of the experiment. The results obtained in this manner are more satisfactory than actual



Graph VII.

readings for comparison of one series with another, excepting at straw temperatures close to the room temperature.

The rise in temperature observed in unsterilised straw under the conditions stated is shown in Graph VII, which is the average of readings taken in six flasks. The deviations from the mean are not large except at the maximum point, the differences probably being accounted for by variations in conditions of packing and, in consequence, of aeration. Furthermore, the peak point was not always reached on the same day though, in general, this was the case. The extent of the deviation close to this point, in the case of six flasks of unsterilised straw, is shown in Table XIII.

It will be seen that, after reaching a peak on the sixth to seventh day, the fall is steady to about 25° C., at which temperature the straw is maintained for about ten days, after which there is a gradual fall to room temperature. The decomposition is, of course, effected in this case not

by fungi alone but by bacteria and Actinomycetes in addition. The highest temperature recorded was 40·2° C.

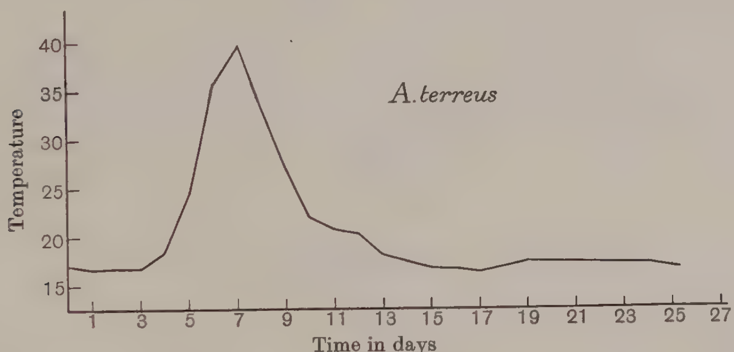
Table XIII.

Heat production in unsterilised straw.

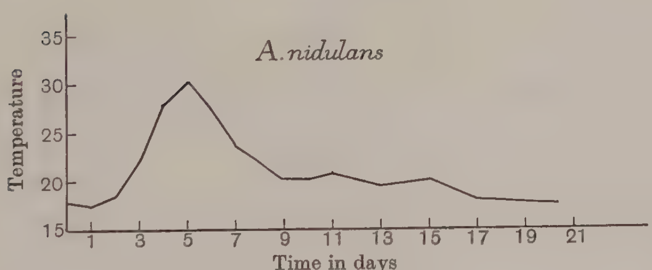
Day	*A*	*B*	*C*	*D*	*E*	*F*	Mean
6	36·4	38·4	32·4	32·4	33·2	40·2	35·0
7	36·1	37·0	36·2	36·2	36·2	39·7	36·9
8	32·9	34·7	31·5	32·9	31·7	37·3	33·5

Single organisms on sterile straw.

When it was desired to test the thermogenic ability of individual organisms or known mixtures of organisms, the technique had to be modified. The straw was sterilised in bottles with mouths of the same

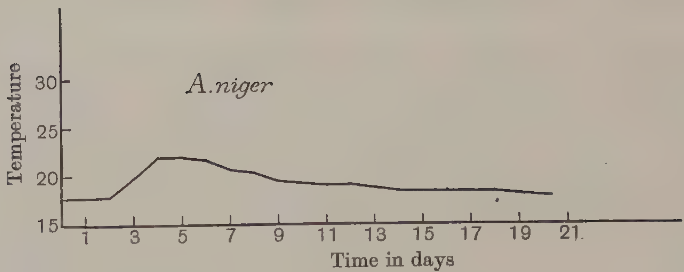


Graph VIII.

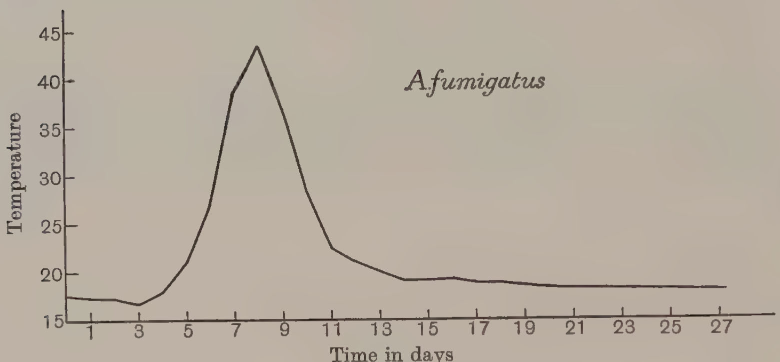


Graph IX.

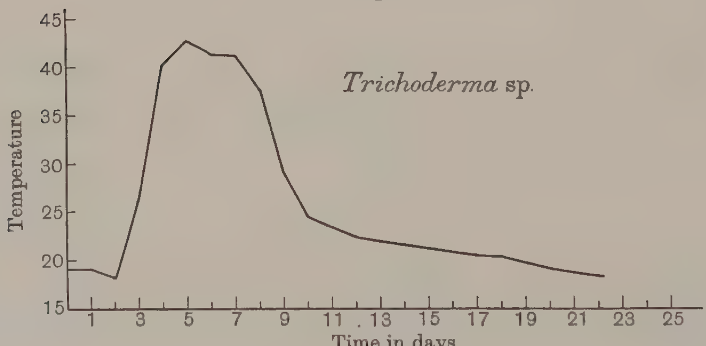
diameter as the vacuum flasks. Four treatments at 115° C. on consecutive days is necessary for complete sterilisation. The vacuum flasks were steamed while open, and the straw transferred from the bottles to the flasks in a pure culture room, after which the flasks with straw were again



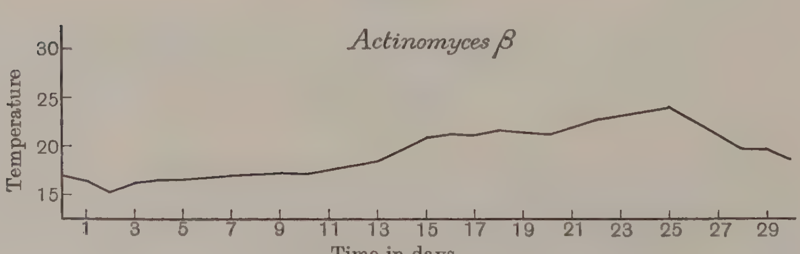
Graph X.



Graph XI.



Graph XII.



Graph XIII.

steamed and plugged in the steam. 1·4 gm. of ammonium nitrate in sterile solution was added to each, together with a heavy inoculum of the spores of the required organism suspended in sterile water, the total volume added being 100 c.c. The inocula were obtained by washing with sterile water slope cultures on Czapek's agar (excepting in the case of *Sepedonium* sp. which is cultured on Waksman's agar) previously incubated at the optimum temperature of the organism concerned. A thermometer was then placed through a cotton-wool plug as before and the flask laid on its side. Platings were made at the conclusion of the experiments to ensure that chance infection had not taken place. Fungal contamination could readily be observed in the single factor experiments by inspection of the rotted straw, since it is found to assume a characteristic colour on decomposition with each of the organisms investigated. Four vacuum flasks were taken in each of the experiments to be described. The averages of the readings taken, corrected for fluctuations in room temperature, are plotted in Graphs VIII to XIII. It will be seen that each of the organisms isolated, with the exception of *Sepedonium* sp. which does not grow alone on straw, is capable of bringing about an increase in temperature of the straw. The mean peaks and maxima are given in Table XIV.

Table XIV.

Heat production of single organisms on sterile straw.

Organism	Mean peak (° C.)	Day	Max. temp. observed (° C.)	Graph
I. *A. terreus*	39·4	7	41·5	VIII
II. *A. nidulans*	30·3	5	33·8	IX
III. *A. niger*	22·2	4	24·1	X
IV. *A. fumigatus*	43·3	8	43·5	XI
V. *Trichoderma* sp.	42·8	5	49·0	XII
VI. *Actinomyces β*	24·3	25	28·2	XIII

In the cases in which a high temperature is attained, the rise observed is very rapid and the maximum maintained for a few hours only. The subsequent fall from the higher temperatures is as rapid as was the rise. When, however, a moderate increase only is caused, both rise and fall are observed to be more gradual.

Of the Aspergilli, both *A. terreus* and *A. fumigatus* (Graphs VIII and XI) gave temperatures over 40° C., the latter being rather more effective in heat production than the former. James, Rettger and Thom (9) record a maximum of 51·5° C. for a strain of *A. fumigatus* on cornmeal in the presence of a constant oxygen supply.

A. nidulans and *A. niger* (Graphs IX and X) were decreasingly less effective in the production of heat, the latter giving only a slight rise with a maximum of 24·1° C. Miehe (13) has reported that *A. niger* gave a temperature of 48° C. in hay, but the methods of sterilisation employed by him render the figures obtained for pure cultures somewhat doubtful.

Trichoderma sp. (Graph XII) was found to be powerfully thermogenic and the highest temperature recorded during this work, namely, 49·0° C., was given by this organism. The heat production is particularly rapid, in one case there being an increment of 30·7° C. in three days. The subsequent fall is rather more gradual than observed in the case of the other organisms. The flasks inoculated with this organism revealed a feature of considerable importance, in that the peak temperatures reached are outside the temperature range of growth of this organism on a synthetic medium. The fungus grows luxuriantly on Waksman's agar, yet no growth could be obtained on this medium at 40° C., in spite of the fact that the organism is capable of raising the temperature of straw considerably above this point. Miehe (13) found that hay often rose in temperature until it was sterile, since chemical oxidation had carried the heating on above the thermal death point of the organisms which were responsible for the initial rise. This, however, did not obtain in the case under discussion, since cultures of the organism were taken from the flask which attained 49° C. It is clear that the properties of this organism on a natural substrate cannot reliably be deduced solely from its behaviour on a particular synthetic medium.

Actinomyces β (Graph XIII) was found to be capable of raising the temperature of the straw to a limited extent, the maximum observed being only 28·2° C. The heating, too, is very much more gradual than that observed with the other fungi, which usually attained or passed the peak by the end of one week from the time of inoculation, whereas the peak with *Actinomyces β* was reached only after twenty-five days. This organism is clearly of a type quite different to the other fungi so far as thermogenic powers are concerned.

Combinations of organisms on sterile straw.

Certain preliminary work has been carried out on the effect of mixed inoculations of the above organisms on heat production in straw. The technique employed was as described previously, excepting that the inocula consisted of a mixed suspension in sterile water of the spores in question in approximately equal amounts.

A. fumigatus was shown above (Graph XI) to produce a higher tem-

perature than the other Aspergilli and, therefore, the effect of combination of this organism with the others was first tested. The results are given in Graphs XIV–XVII, and the mean peaks and maximum temperatures in Table XV below. Four flasks were employed in each case.

Table XV.

Heat production of A. fumigatus *in association with other organisms.*

Organism	Mean peak (° C.)	Day	Maximum (° C.)	Graph
I. *A. fumigatus*	43·3	8	43·5	XI
II. *A. fumigatus + A. terreus*	39·7	6	41·8	XIV
III. *A. fumigatus + A. nidulans*	32·4	5	33·1	XV
IV. *A. fumigatus + A. niger*	26·2	5	31·5	XVI
V. *A. fumigatus + Actinomyces β*	34·6	8	37·1	XVII

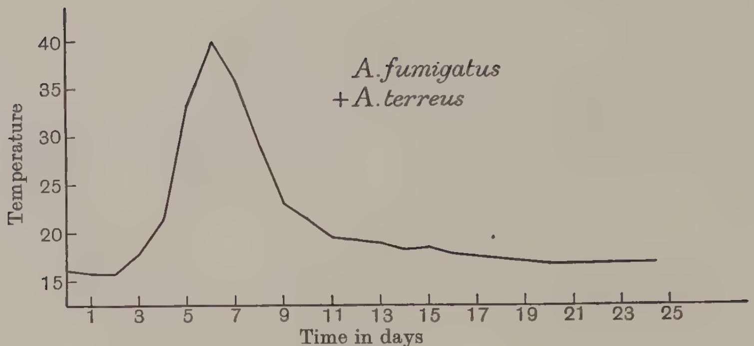
For reasons which will be given later, it is not possible to generalise on the effect of association of one organism with another, and the organisms tested must in each case be quoted. It will be seen that the association of *A. fumigatus* with another organism in each case resulted in the production of a temperature lower than that given by *A. fumigatus* alone. Furthermore the temperature produced is much nearer to that given by the associated organism alone than that by *A. fumigatus*. In pure culture *A. terreus* gives a temperature of 39·4° C. and with *A. fumigatus* 39·7° C., *A. nidulans* alone 30·3° C., but with *A. fumigatus* 26·2° C. In association with Actinomyces the position is rather different. A temperature of 34·6° C. is attained on the eighth day, and there is a complete absence of a slow and steady rise to a peak about the twenty-fifth day, as given by the Actinomycete alone. The Actinomycete had nevertheless developed extensively as revealed by an examination of the rotted straw. Association with the fungus had apparently reduced its thermogenic power.

This apparent loss of thermogenic power was observed also in cases in which *Actinomyces β* was combined with other organisms. Certain of these are quoted below in Table XVI.

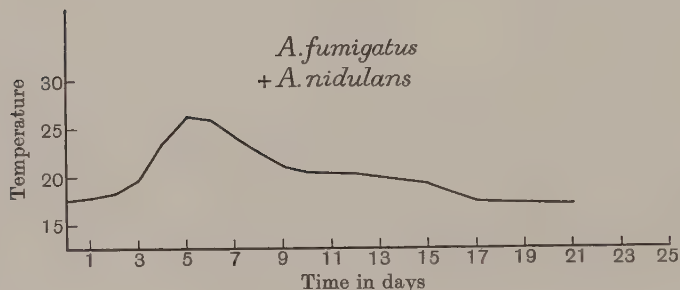
Table XVI.

Heat production of Actinomyces β *in association with other organisms.*

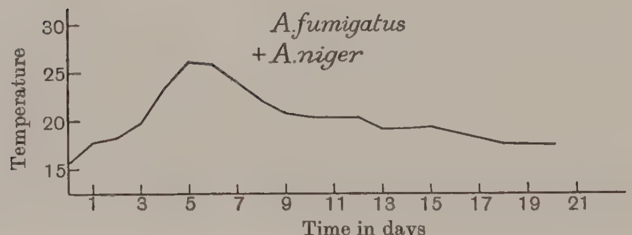
Organisms	Mean peak (° C.)	Day	Maximum (° C.)	Graph
I. *Actinomyces β*	24·3	25	28·2	XIII
II. *Actinomyces β + A. terreus*	32·7	6	35·1	XVIII
III. *Actinomyces β + A. terreus + A. fumigatus*	29·3	5	31·2	XIX



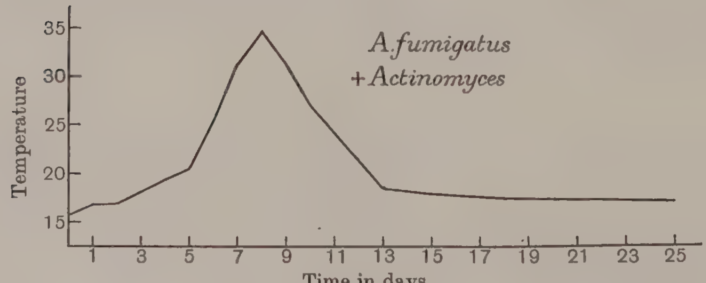
Graph XIV.



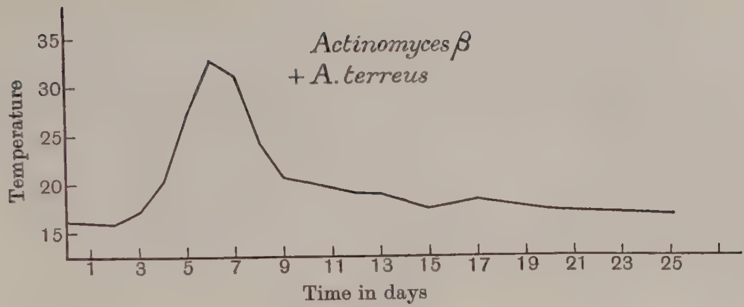
Graph XV.



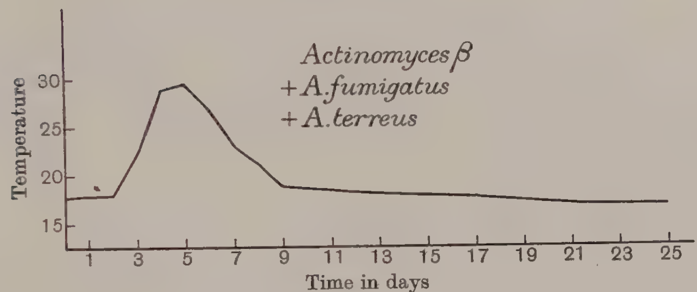
Graph XVI.



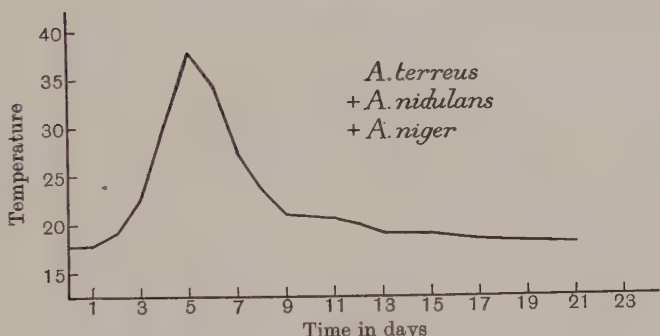
Graph XVII.



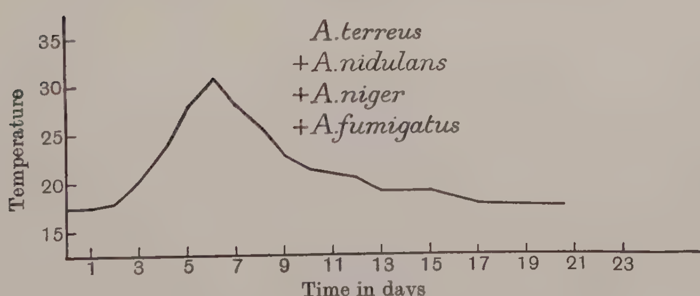
Graph XVIII.



Graph XIX.



Graph XX.



Graph XXI.

Not only is the typical peak for *Actinomyces β* not shown, but the presence of this organism results in the production of less heat, since *A. terreus* alone gave a mean peak of 39·5° C. but with the Actinomycete only 29·3° C.

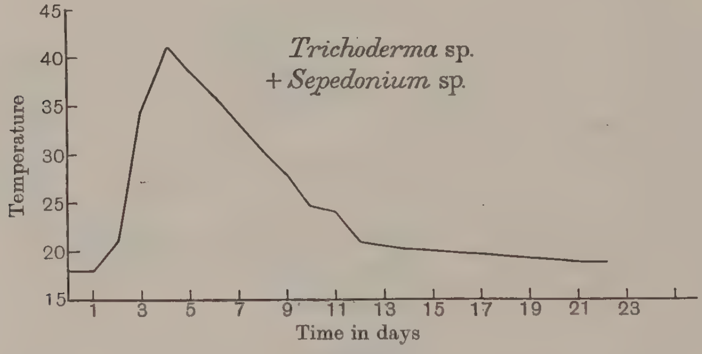
This depressing effect of association does not appear to hold in all cases, as is shown in Table XVII.

Table XVII.

Heat production of certain Aspergilli in association.

Organisms	Mean peak (° C.)	Day	Maximum (° C.)	Graph
I. *A. terreus* + *A. nidulans* + *A. niger*	37·6	5	38·6	XX
II. *A. terreus* + *A. nidulans* + *A. niger* + *A. fumigatus*	30·6	6	31·9	XXI

The temperature given by *A. terreus* together with *A. niger* and *A. nidulans* is very little below that of *A. terreus* alone, and much above that given by either *A. niger* or *A. nidulans* alone. When, however, the straw is inoculated with *A. fumigatus* in addition to these three Aspergilli the temperature reached is lower, being only 30·6° C.



Graph XXII.

The effect of association of *Sepedonium* sp. on *Trichoderma* sp. was investigated since, although the former organism is not a cellulose decomposer, it appears to occur to a considerable extent in rotting straw. Moreover its optimum temperature is approximately that attained by straw inoculated with *Trichoderma* sp. alone. If *Sepedonium* sp. assists in the decomposition of straw, perhaps by removal of degradation products or transitory substances produced by other organisms, it was thought that in association with *Trichoderma* sp. it might be responsible

for an even higher temperature. This, however, was found not to be the case. The figures are given in Table XVIII and Graph XXII.

Table XVIII.

Heat production of Trichoderma *and* Sepedonium.

Organisms	Mean peak (° C.)	Day	Maximum (° C.)	Graph
I. *Trichoderma* sp.	42·8	5	49·0	XII
II. *Trichoderma* sp. + *Sepedonium* sp.	40·8	4	43·5	XXII

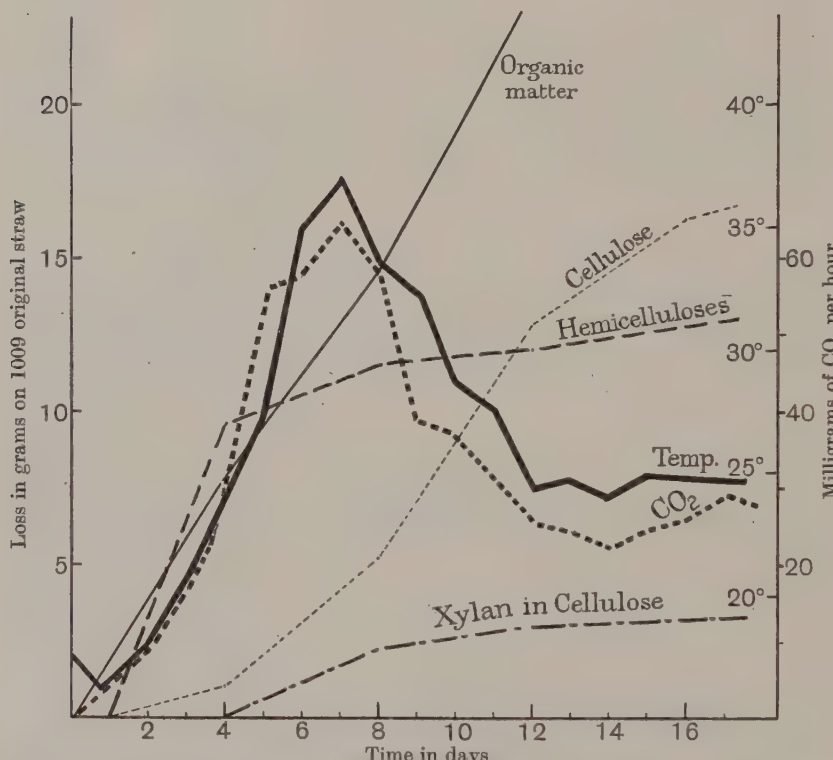
If there is any significance in the results, the association of the two gives a temperature rather lower than *Trichoderma* sp. alone, in which case also the high temperature is maintained for about two days. In the presence of *Sepedonium* sp., however, the fall is much more rapid.

Relation between evolution of CO_2 *and thermogenesis.*

It was thought to be of interest to determine the relation between heat production and rate of decomposition as evidenced by the evolution of CO_2, since the two processes are not of necessity parallel.

Unsterilised straw was rotted as before in a vacuum flask and a slow stream of CO_2-free air (rate = 2 l. per hour) drawn through. The CO_2 evolved was absorbed by baryta in a Pettenkoffer tube in the usual manner. The CO_2 evolved is plotted as milligrams per hour against time. The temperature of the straw in the flask is lowered not inconsiderably by the cooling effect of the air stream, so that in Graph XXIII in which the CO_2 production is plotted, the temperature curve given is the mean from six non-aerated flasks. It is true that the conditions are not precisely similar, but the effect of aeration on the decomposition will merely be to speed up the process. It will be seen that the temperature rise and fall follows very closely the production of CO_2. When fungal activity is at its peak as measured by the production of CO_2 so, also, is the evolution of heat. More striking, however, is the fact that there is such a marked peak in CO_2 production, since the loss of organic matter is very little, if at all, greater over the period of the fourth to the eighth day, when CO_2 and heat production are at a high figure, than from the eighth to the twelfth day, when they appear considerably lower. This points in the first period to the rapid oxidation of some constituent or constituents with the evolution of much heat, which change is replaced in the second period by an oxidation less complete and yielding a much smaller quantity of heat. On the same graph is plotted actual losses in grams of some of the major constituents of straw. In this preliminary work determinations

have as yet only been made at intervals of four days, but in later studies this period will be shortened. It is the hemicelluloses of the straw that suffer the greatest loss in the opening stages of the decomposition. The approximate loss of hemicellulose is given on the same graph. In four days 100 gm. of straw loses nearly 10 gm. of hemicellulose, while by the eighth day the loss is 11·5 gm. and, in addition, there is a loss of nearly

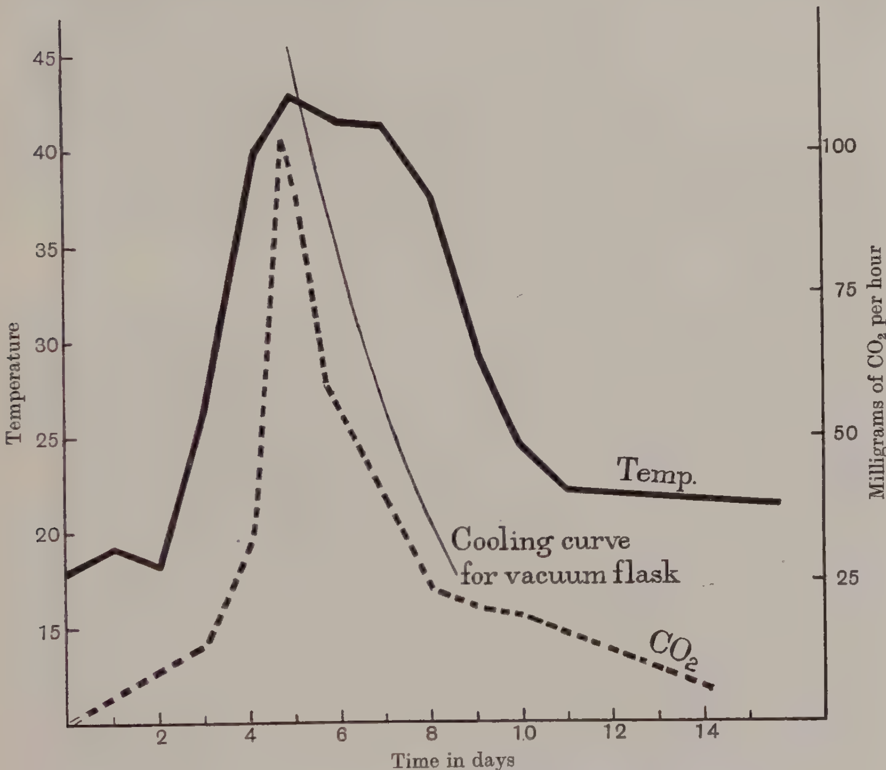


[1]Graph XXIII. Oat straw unsterilised. Temperature, CO_2 yield and loss of constituents.

2·5 gm. of the xylan associated with the cellulose. Over the same period of eight days only 5 gm. of cellulose is lost, 4 gm. being removed between the fourth and eighth day. Up to the peak point of heat production and CO_2 evolution on the seventh day, there has been a loss of 11 + 2 gm. of hemicellulose material and 4 gm. approximately of cellulose. After the

[1] The apparent loss of the constituents of straw frequently appears greater than the actual loss, owing to partial decomposition of some constituents. Cellulose and hemicelluloses may be so attacked that they no longer appear as cellulose or hemicellulose in the analyses, but nevertheless have not been completely oxidised away to CO_2 and water.

eighth day the loss of cellulose increases steadily in rate and, in the next eight days, 11 gm. is lost but, nevertheless, the temperature and CO_2 evolution fall. It would appear, therefore, that the decomposition of hemicellulose material is accompanied by the evolution of a considerable amount of heat, while in the decomposition of cellulose much less is liberated.



Graph XXIV. *Trichoderma* sp. Heat production and CO_2 evolved.

The observations just recorded are for a mixed flora of fungi and bacteria. It remains for further work to show whether in pure cultures the decomposition is similar in order. The CO_2 evolution of *Trichoderma* sp. has been tested in pure culture. The technique adopted was as described above, with the exception that CO_2 was removed from the air stream by concentrated potash and over soda lime, after which it was passed through concentrated sulphuric acid and sterile water before being led through the vacuum flask. The CO_2 evolution (Graph XXIV) exhibits a very sharp rise about the fifth day, at the same time at which heat production

is at its maximum. The subsequent fall is, however, almost equally sharp, not paralleled by the temperature records. It should be pointed out, however, that in each case the fall of temperature as recorded is less sharp than must actually occur. Were there no further heat production in the flask once it had attained 45° C., an examination of the cooling curve for the flask shows that two days would elapse before the temperature fell below 25° C. Actual heat production on the down-grade portion of the peaks is, therefore, masked by heat conservation by the vacuum flasks. An apparatus is being devised which will give a true, not a fictitious record, on this portion of the curve. Nevertheless, it would appear from the work described that it is likely that a close relationship will be found between evolution of heat and CO_2 production and, further, that heat production in straws may not improbably be connected with the decomposition of the hemicelluloses.

Discussion of thermogenesis.

As outlined in the introduction, the decomposition of straws is the assimilation of certain of its constituents by the micro-organisms concerned. The carbonaceous materials are chiefly employed as a source of energy, and to a lesser extent for structural purposes. Nitrogenous substances are in demand for conversion to microbial protein, and the nitrogen supply is frequently the factor limiting the extent of development of the organisms. The carbon compounds of the straw are almost solely in the form of complex organic substances of high molecular weight. These are degraded by the organism to substances of simpler constitution and, finally, to CO_2 and water. Some of the stages may take place outside of the organisms by the hydrolytic action of exo-enzymes secreted by them. Later stages are brought about by the process of respiration within the cell. Potential chemical energy for the substances assimilated is liberated during this process partly as kinetic energy, partly as heat energy and, in the case of an actively growing organism, partly transformed in synthesis to potential energy in another compound. The efficiency of energy utilisation varies considerably from organism to organism. By many workers fungi have been stated to be able to transform, under favourable conditions, only about one-half of the carbon source into fungal tissue while, under less favourable conditions, the amount is considerably reduced. That is to say only 50 per cent. or less of the available energy is utilised directly by the organism. Aerobic bacteria commonly utilise only 10–25 per cent. of the available energy. The energy not utilised may be in one or two forms. Either it is as

potential chemical energy in the form of intermediary products not completely oxidised, or it is liberated as heat, or both. Such heat production, or microbial thermogenesis as it was termed by Cohn (2), is clearly, then, an ancillary process in the metabolism of the micro-organisms concerned. It may nevertheless be, in effect, one of great importance, since the rise in temperature produced may markedly affect the rate of decomposition. The temperature of the substrate may be brought by this means to the optimum temperature of the organisms decomposing it, and the decomposition in consequence will be accelerated. Furthermore, the rise in temperature in unsterilised materials will bring about a modification in type of the active flora. Thermophilic forms will replace the normal type of organism over, perhaps, 45° C., and the course of the decomposition may be altered by their action.

The thermogenic ability of any organism varies from one source of carbon to another and, similarly, the amount of heat which may be liberated during the degradation of a given carbonaceous material varies from organism to organism. Since microbial thermogenesis is a subsidiary effect in metabolism, the amount of heat liberated in the decomposition of a material such as straw is not, therefore, of necessity, any measure of the extent of its decomposition. Decompositions of equal extent effected by two organisms separately may, therefore, result in the liberation of widely different amounts of heat energy. If the assumption is made that each of the constituents of the straw is available to a similar degree to two organisms, one with marked thermogenic powers, and the other with little or no thermogenic ability, then, on inoculation with a mixture of the two, an intermediate temperature should result since precisely half the constituents would be utilised by each. Similarly, if there is one constituent of the straw which may be degraded in two ways by two different organisms, one route producing heat and the other not, then on association of the two the factor which determines the amount of heat produced, other things being equal, is the availability of that constituent to each of the organisms. If it is of equal availability, then the temperature produced will be a mean value, but if of unequal availability, then the balance will be thrown to one side or the other. The element of competition is involved. Information bearing on the reasons for competition can only be obtained by biochemical studies of the changes brought about by the organisms concerned, both individually and in association.

However, the association of micro-organisms does not necessarily always involve competition, for their action is in many cases not

competitive but co-operative. Certain stages in a particular decomposition may be more effectively and rapidly carried out by one organism than another, or intermediary products produced by one organism may be utilised readily by another. In this way heat may be produced rapidly from substances which might otherwise be decomposed only slowly or not at all. Moreover, general decomposition may proceed further in co-operative association owing to the removal of by-products. It is possible, therefore, that certain associations of organisms may be of greater thermogenic power than the individuals participating, since the decomposition may proceed by a different route. The natural decomposition of plant tissues should probably be viewed in the aggregate as a co-operative decomposition of a high order. No single organism that has been isolated is capable of effecting so extensive a decomposition as a mixed flora. It is probable that the association of organisms of different types with different metabolic cycles and affinities is of great importance. Bacteria and fungi working together are, in general, peculiarly efficient, and this is easily explained since their metabolic processes as revealed by the efficiency of utilisation of energy are of a different order. In the experiments described above, the organisms tested in combination were, in practically all cases, closely allied in type and with similar properties. For these reasons, therefore, it is not surprising that so far as thermogenesis is concerned, a competitive effect was noticed.

The effect of association of one organism with another is a field but little explored. Savastano and Fawcett (21) have recently examined the extent of decay produced in citrus fruits inoculated with known mixtures of fungi. Certain mixtures gave increased rates and extents of decay, while others had a depressing effect. Since their results are based on measurements and physical observations alone, without any chemical investigations of the changes involved, no explanation of the effects of association can be offered, and their results are merely empirical.

Biochemical methods alone can give any explanation of the observed effects of association, and it is along these lines that it is proposed to develop the work described in this paper. Attempts are being made to determine the metabolic affinities of some of the organisms active in the decomposition of plant materials, and the interaction of these on association will then be examined.

IV. SUMMARY.

1. Since the major part of the loss of organic matter in the biological decomposition of mature plant materials is accounted for by the loss in cellulose, the present investigation was directed to the nature of the fungi assisting in this process.

2. Certain fungi were isolated, which though actively attacking cellulose in straws, do not make more than meagre growth on cellulose-agar plates.

3. All were found to have their optimum temperature above that usual for fungal growth; three, indeed, could develop at 50° C.

4. The availability of various nitrogen and carbon compounds to these organisms was tested. Particular attention was directed to those sugars naturally occurring in that class of substances known as hemicelluloses. The pentose sugars, though readily available, did not appear so suitable as their sterically related hexoses.

5. Variation, in the form of "sectoring" of the colonies, was observed in the case of one organism only, namely *Sepedonium* sp. A wedge of a light-spored variant appeared, which has remained constant throughout several culture generations. Certain carbohydrates, xylose and fructose, in particular, favoured the appearance of this variant.

6. Since the decomposition of straw and other plant materials by a mixed microflora is often accompanied by a rise in temperature, the thermogenic power on sterile straw of the organisms individually was examined. A considerable rise in temperature was observed in some cases, and some rise in all. The highest temperature attained was 49·0° C., which is outside the temperature range on a synthetic medium of the organism giving it, namely *Trichoderma* sp. That this was not due to chemical oxidation following the rise due to fungal activity was shown by the fact that sub-cultures of the organism were obtained from the straw which had reached this high temperature.

7. The thermogenic powers of certain simple combinations of these organisms were tested, and in each case a depressing effect was observed. In the cases in which two organisms only were involved, the combination gave a temperature near to that of the one with lesser thermogenic power.

8. The evolution of CO_2 closely paralleled the production of heat, when the heat-retaining ability of the vacuum flasks is taken into account.

9. The period of maximum heat production was shown to correspond with that of rapid loss of hemicelluloses. Cellulose decomposition, which

is the next phase, does not appear to result in the production of much heat.

10. Although microbial thermogenesis is an incidental, and not an essential effect of metabolism, it may markedly affect a decomposition by bringing the substrate to the optimum temperature, or even by modifying the flora involved.

11. The association of organisms may be competitive or co-operative in thermogenic power. In *competitive association* there is competition for constituents of similar availability, and the heat produced by organisms of different thermogenic power in competitive association will be an intermediate figure. In *co-operative association* certain stages in the decomposition may be more effectively and rapidly carried out by one organism than another, or intermediate or by-products readily utilised. In this way the decomposition may be more rapid and more complete, and heat may be produced from substances which might otherwise not be attacked. Certain associations of organisms may, therefore, be of greater thermogenic power than the individuals participating.

The author is indebted to Sir John Russell, F.R.S., Director of the Rothamsted Experimental Station, for placing at his disposal the facilities of that Station, and to the Department of Scientific and Industrial Research for Senior Research Award, during the tenure of which this work has been carried out.

Especially are his thanks due to Dr W. B. Brierley, Head of the Department of Mycology, whose suggestions and advice throughout have been invaluable.

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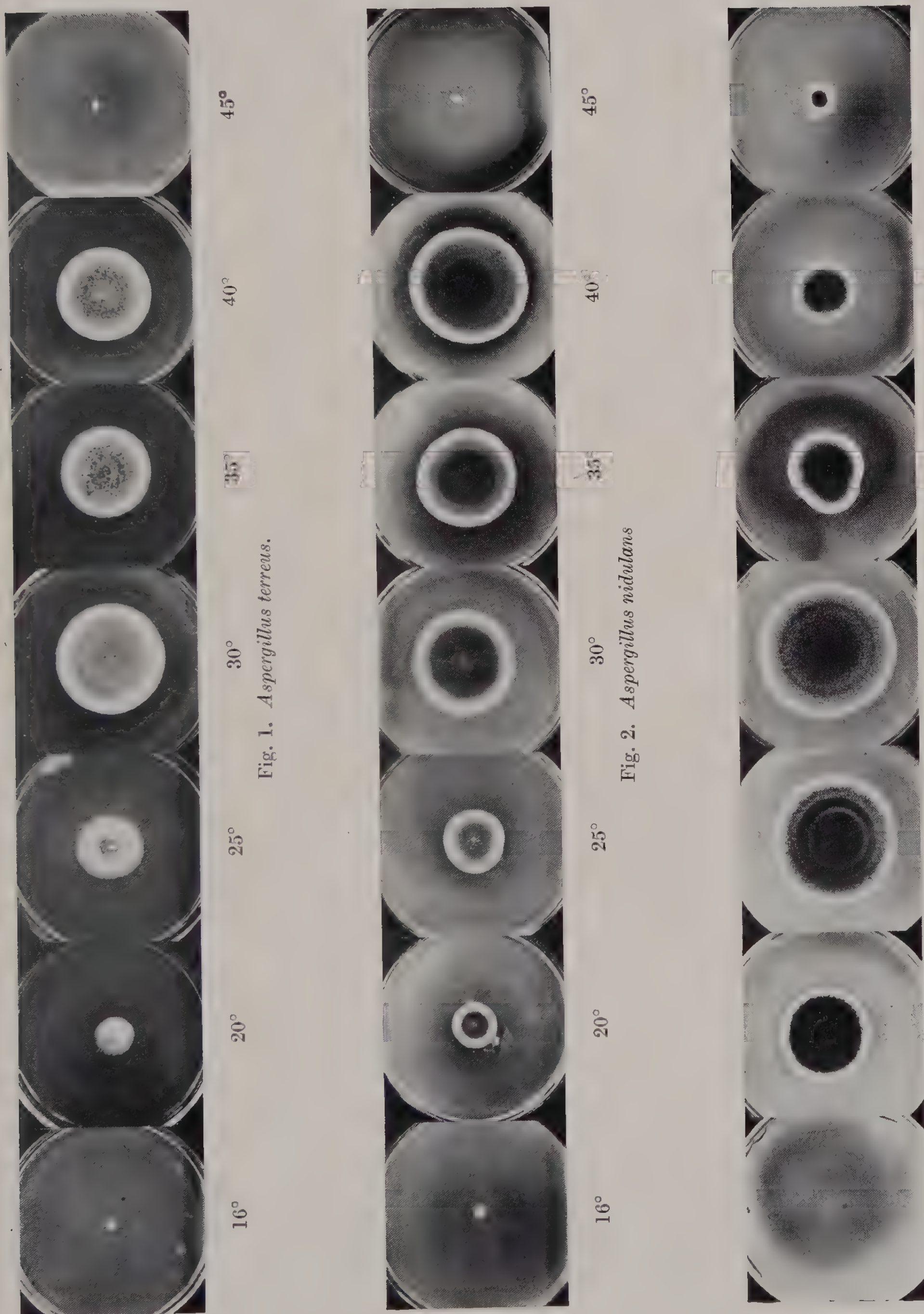


Fig. 1. *Aspergillus terreus.*

Fig. 2. *Aspergillus nidulans*

Fig. 3. *Aspergillus niger*

NORMAN.—The Biological Decomposition of Plant Materials (pp. 575–613).

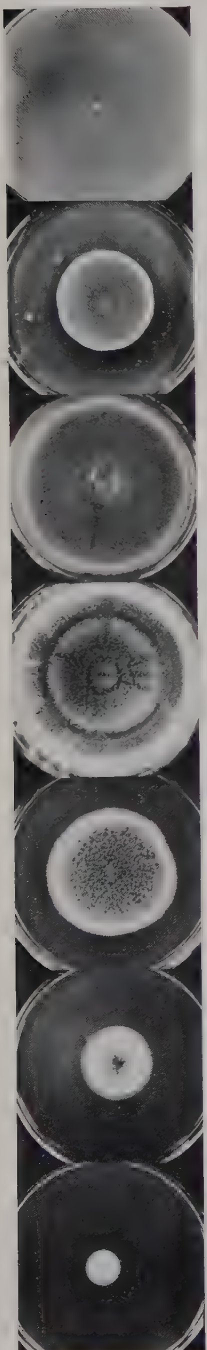


Fig. 1. *Aspergillus fumigatus*

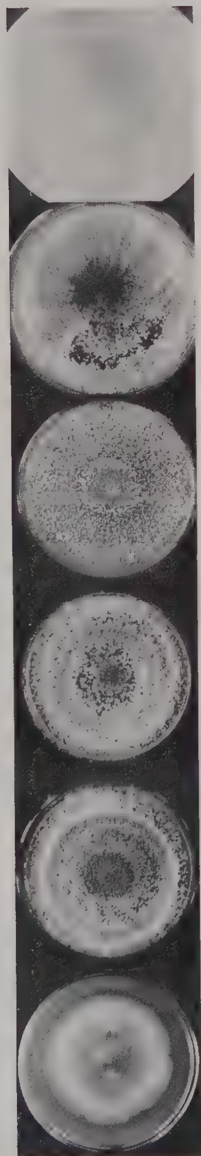


Fig. 2. *Trichoderma* sp.

Fig. 3. *Sepedonium* sp.

NORMAN.—THE BIOLOGICAL DECOMPOSITION OF PLANT MATERIALS (pp. 575–613).

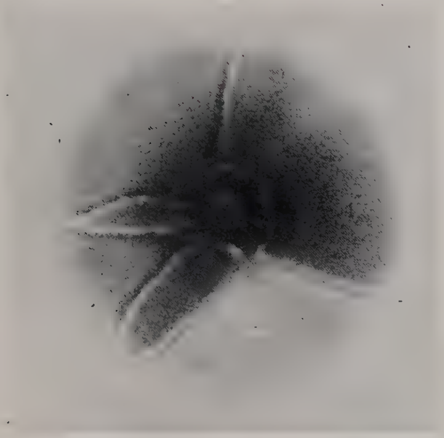
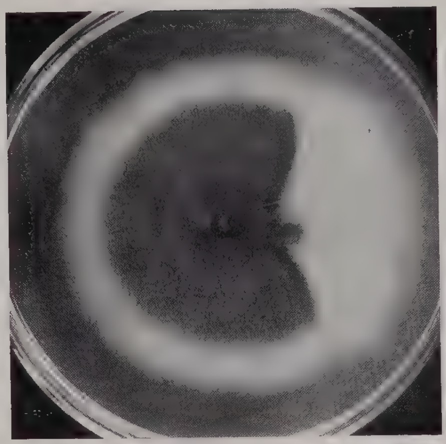


Fig. 1.



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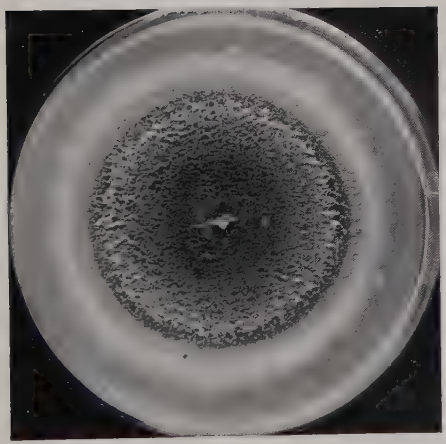

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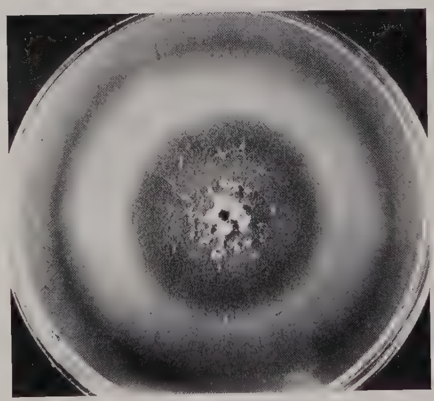

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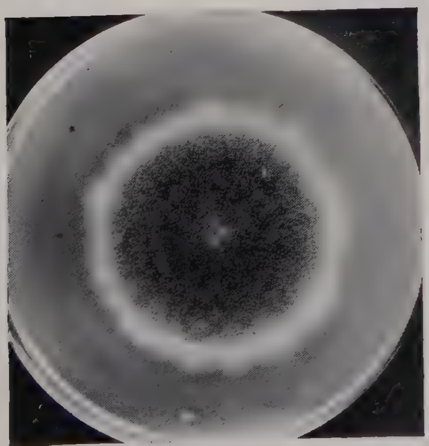

Fig. 5.

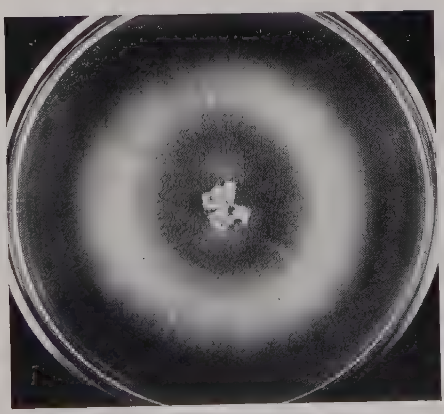

Fig. 6.

Sepedonium sp. on various media.

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EXPLANATION OF PLATES XXXVII—XXXIX

PLATE XXXVII.

Fig. 1. *Aspergillus terreus.* Six days' growth on Waksman's agar (pH = 4·0).
Fig. 2. *Aspergillus nidulans.* Six days' growth on Waksman's agar.
Fig. 3. *Aspergillus niger.* Six days' growth on Waksman's agar.

PLATE XXXVIII.

Fig. 1. *Aspergillus fumigatus.* Six days' growth on Waksman's agar.
Fig. 2. *Trichoderma* sp. Six days' growth on Waksman's agar.
Fig. 3. *Sepedonium* sp. Six days' growth on Waksman's agar.

PLATE XXXIX.

Fig. 1. *Sepedonium* sp. on fructose-peptone-agar showing sectoring.
Fig. 2. *Sepedonium* sp. on xylose showing sectoring.
Fig. 3. *Sepedonium* sp. (dark or normal strain) on maltose-peptone-agar.
Fig. 4. *Sepedonium* sp. (light variant) on maltose-peptone-agar.
Fig. 5. *Sepedonium* sp. (dark or normal strain) on xylose-peptone-agar.
Fig. 6. *Sepedonium* sp. (light variant) on xylose-peptone-agar.

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THE RELATION BETWEEN THE NUMBERS OF A SOIL BACTERIUM AND THE AMMONIA PRODUCED BY IT IN PEPTONE SOLUTIONS; WITH SOME REFERENCE TO THE EFFECT ON THIS PROCESS OF THE PRESENCE OF AMOEBAE

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(With 6 Text-figures.)

Though the decomposition of nitrogenous materials by bacteria, with the accompanying liberation of ammonia, has been extensively studied in general, yet comparatively little detailed work has been done on this process as it takes place in the soil, compared with the attention that has been given to other phases of the nitrogen cycle, such as nitrogen fixation.

A great deal of work on "ammonification" in the soil has been done from the point of view of the availability of various commercial products as nitrogenous fertilisers; for instance, the studies by Lipman and his co-workers (11); in this work, as in most of the earlier work, no attempt was made to simplify the problem by considering a section only of the soil population.

The work on ammonification in soils before 1923 has been reviewed in detail by Waksman (20); he records the observation that higher bacterial numbers in a soil do not necessarily coincide with a higher formation of ammonia in that soil compared with other soils.

Another interesting observation on bacterial numbers is that of Beckwith, Vass and Robinson (2); they compared the amount of ammonia formed in ten days, and the number of bacteria present at the end of that time, in six different soils at different moisture contents. They state that "the greater the number of bacteria per gram of soil, the less the amount of ammonia produced per number," but they base this statement on single observations, and make no attempt to follow the course of ammonification.

Detailed studies of ammonia formation by pure cultures of soil micro-organisms have been made by Waksman (19) and Waksman and Lomanitz (21), in which the course of ammonification was followed over a period of time, but these studies are mostly on soil fungi, and though soil bacteria are included among the species studied, their numbers are not considered. Then, too, these and similar studies on ammonification are concerned as much with the "protein-sparing" action of carbohydrate as with any other aspect of ammonia formation, thereby following the work of Kendall and his co-workers with pathogenic bacteria (10).

Robinson and Tartar (16), who worked with pure species of soil bacteria in sand cultures with various sources of nitrogen including peptone, observed that the rate of ammonia formation slowed down before nearly all of the nitrogen present in the medium had been converted to ammonia. They let the formation of ammonia proceed to its furthest point, then they sterilised a portion of their medium and re-inoculated it. They obtained very little further output of ammonia, and concluded that there is a part of the protein molecule which resists attack by bacteria, and is incapable of being converted by them into ammonia.

As regards former work on the influence of protozoa on ammonification, reference may be made to the work of Waksman (18) and Skinner (17); neither of these workers, however, attempted to follow the course of ammonia formation by consecutive observations, but was content to record the differences in ammonia content at the end of long periods of incubation.

The experiments recorded in the present paper were carried out with pure cultures of a soil bacterium and a soil amoeba, using a peptone solution, either as a liquid medium, or as the food solution in a sand medium.

Following on the work of Cutler and Crump on carbon dioxide production by bacteria in the presence and absence of amoebae (5), some preliminary experiments were set up to investigate the production of ammonia under similar conditions. These experiments, which were all carried out with sand cultures, had to be discontinued on account of the dying out of the stock of amoebae; they are briefly reported in Part II of this paper.

As the presence of protozoa is known to affect bacterial numbers, it was felt that an investigation of the relation between bacterial numbers and ammonia production by bacteria in the absence of amoebae would be a useful preliminary to future experiments on the effect of protozoa on this process.

The experiments described in Part I of this paper were therefore set up, using the same species of bacteria as in the sand experiments, but in liquid cultures.

I. LIQUID CULTURES.

Methods.

Outline of method. Sixteen portions of a peptone medium were inoculated in pairs with bacteria, one of each pair receiving about ten times the number of bacteria inoculated into the other. The numbers of bacteria in each resulting culture were counted daily, and the amount of ammonia in a sample from each culture was estimated at intervals of a week.

Pure cultures of the soil bacterium "YB" (4) were used, grown on agar slopes, a 48-hour-old culture being employed in all but two cases, where the cultures used were three days old (Cultures 3 and 4). The medium used was a 0·5 per cent. solution of peptone (bacteriological, B.D.H.) in a mineral salt solution of the following composition; NaCl 0·06 per cent., KCl 0·001 per cent., $CaCl_2$ 0·002 per cent., $MgSO_4$ 0·001 per cent., made up in ammonia-free distilled water. The total nitrogen content of this medium was 0·716 mgm. per c.c. 50 c.c. portions of the medium were placed in 250 c.c. conical flasks, and steamed for one hour; the pH was then adjusted to 7·2 with $N/10$ sodium hydroxide, and the flasks steamed again for one hour.

The method of inoculation employed was as follows: a suspension of three or four loopfuls of the "YB" culture was made in 20 c.c. of sterile peptone medium, and one flask was inoculated with 2 c.c. of this suspension by means of a pipette. A second flask was then inoculated with 2 c.c. of a suspension prepared by diluting the original suspension ten times with the peptone medium.

The first series of flasks (Cultures 1, 3, 5, etc.) inoculated with the undiluted suspension, received an average inoculum of 1341 million bacteria in all, as against an average of 159 million bacteria inoculated in the diluted suspension (Cultures 2, 4, 6, etc.). The numbers inoculated were estimated by a haemocytometer count on each suspension, which was checked by a second count on the inoculated liquid in the flask.

The flasks were kept at room temperature; the first ten flasks inoculated (referred to as the "non-aerated" series) were plugged with cotton-wool, but not otherwise aerated. In the other six cases (referred to as the "aerated" series) a slow stream of air, filtered through cotton-wool, was drawn through the liquid by an aspirator; the outgoing air was passed

through an acid trap to recover any ammonia that might be carried over by the air stream, but the amount of ammonia so carried over was found to be negligible.

The bacteria in each flask were counted daily with a Thoma haemocytometer, duplicate loopfuls being taken from each culture. The numbers of bacteria are recorded as millions per c.c. of medium.

A blank estimation of ammonia was made on the medium before inoculation in every case, and after inoculation the ammonia content of each culture was determined at weekly intervals. The method used was that of Woolf (22). Two samples of 1 c.c. each were used in each determination, the ammonia being driven off by aeration in presence of saturated potassium carbonate solution, and caught in 3 per cent. boric acid containing 10 c.c. 0·2 per cent. brom-cresol green per litre, and titrated directly with 0·005 N sulphuric acid made up in the same boric acid-brom-cresol green solution. Each c.c. of acid was equivalent to 0·0854 mgm. NH_3. The titrations were carried out, using a microburette, and in nearly every case the duplicate samples gave a difference in titration of less than 0·05 c.c. The concentration of ammonia is recorded as mgm. NH_3 per c.c. of medium.

The longest time that any culture was kept was seven weeks; several were discarded earlier, either because of contamination or because the bacteria were so clumped together as to make accurate counting impossible.

RESULTS.

The nature of the results obtained can be illustrated by considering two typical cultures, numbers 7 and 8. These two cultures were started at the same time, Culture 7 being inoculated with ten times as many bacteria as Culture 8 (694 million and 66 million per c.c. of inoculum), and were both kept for the full period of the experiment, namely, seven weeks. They were both in the "non-aerated" series, *i.e.* those kept in flasks stoppered with cotton-wool.

Culture 7. (Fig. 1.)

The bacterial numbers in this culture are seen to fall slightly during the first three days; this is followed by a rapid rise to over 600 million per c.c. on the twelfth day, then a slight drop, followed by a rise to 900 million at the end of the third week, and another rise to nearly 1200 million at the end of the fourth week; after this the numbers fluctuate about 900 million per c.c.

Considering the amount of ammonia present per c.c., it is seen that this increases slowly during the first week, more rapidly during the second week, and thereafter the rate of increase falls off continuously.

The nature of this curve of ammonia production will be discussed later, but at present it is sufficient to note, firstly, that the formation of

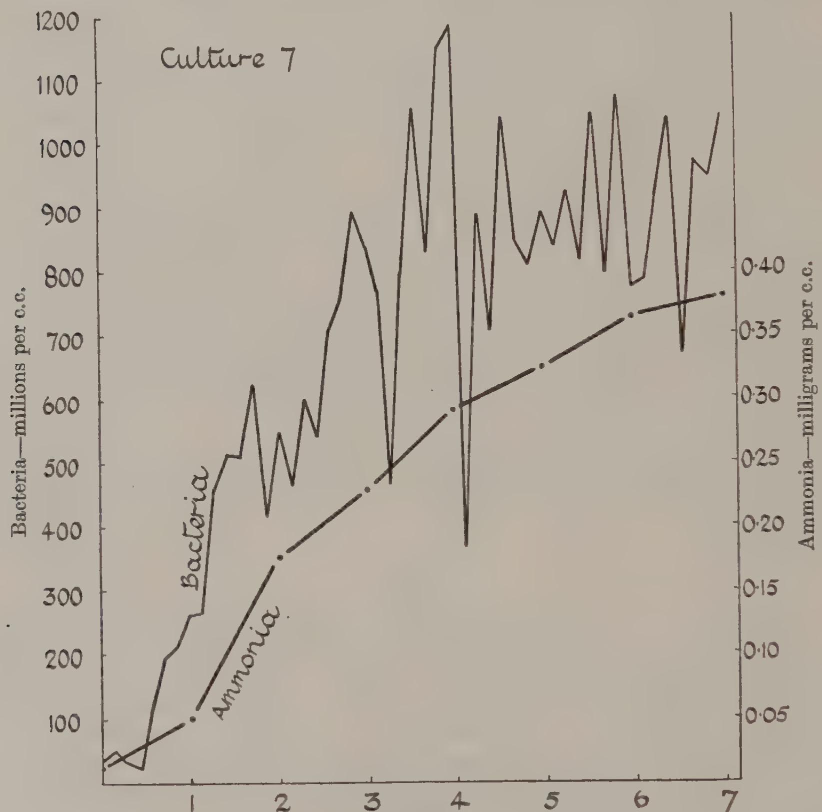


Fig. 1.

ammonia is extremely slow; as the amount of ammonia present is still increasing at the end of seven weeks, the process is evidently not complete; and, secondly, that the theoretical end-point of the reaction deduced from the curve is in the neighbourhood of 0·4 mgm. ammonia per c.c., which corresponds to less than 50 per cent. of the total nitrogen present (47·5 per cent.).

Culture 8. (Fig. 2.)

In this culture the bacterial numbers remain at a low level, below 40 million per c.c., for nearly two weeks; then a slight rise at the beginning of the third week is followed by an extremely rapid rise to nearly 800 million, at twenty-two days. The maximum number, 850 million per c.c., is reached at the end of the fourth week, and the bacterial numbers then fall off, with fluctuations, to an average of about 470 million in the seventh week.

The amount of ammonia present per c.c. shows no increase at all in the first week, and only a very small increase (0·01 mgm.) in the second; there is a greater increase in the third week, and still greater in the fourth, and then the rate of increase falls off continuously.

On comparing these results with those obtained from Culture 7, it will be seen that there is a conspicuous retardation, both of bacterial growth and ammonia production, during the first two weeks. The bacterial numbers in Culture 8 then follow roughly the same course as those in Culture 7, though at a lower level.

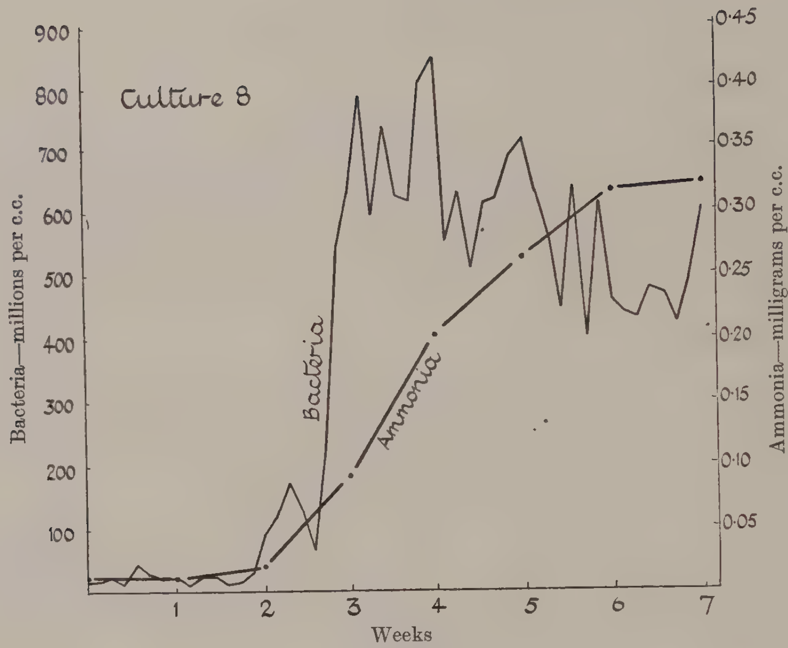
In spite of the great retardation in ammonia production in Culture 8 compared with Culture 7, once the reaction does start it proceeds with as great a velocity; the amount of ammonia formed in four weeks from the start of the reaction (which is taken as the end of the second week after inoculation in the case of Culture 8) is 0·297 mgm. per c.c. in Culture 8, as against 0·281 mgm. in Culture 7.

The results obtained from the other cultures are similar to these, and will not be discussed in detail. The retarding effect of the diluted inoculum was observed in every case.

Effect of aeration. (Fig. 3.)

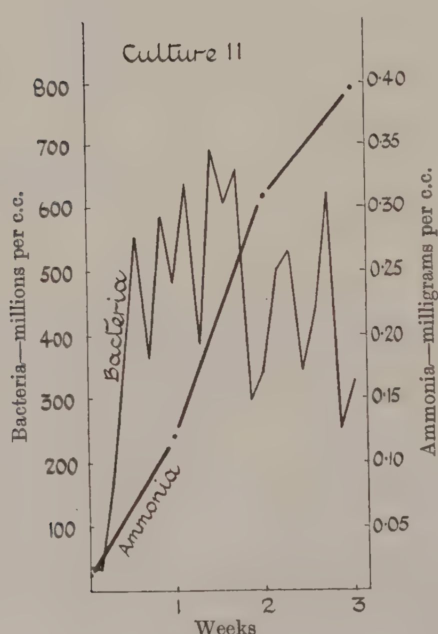
It will be noticed in the description of the methods used that the last six cultures to be set up were kept with a slow current of air passing through the liquid. There were certain differences between these "aerated" cultures and the cultures kept in stoppered flasks ("non-aerated").

The "aerated" cultures showed a much quicker initial increase in bacterial numbers; the initial "lag" period in these cultures which received a large inoculum is reduced from three or four days to one or two days, and by the fourth day the numbers have reached 400 millions per c.c. or more. The average bacterial numbers over the whole period, however, are no greater than in the "non-aerated" flasks over the same period. Corresponding to this greater initial increase in bacterial numbers,



Culture 8
Bacteria
Ammonia
Bacteria—millions per c.c.
Ammonia—milligrams per c.c.
900
800
700
600
500
400
300
200
100
0·45
0·40
0·35
0·30
0·25
0·20
0·15
0·10
0·05
1
2
3
4
5
6
7
Weeks

Fig. 2.



Culture 11
Bacteria
Ammonia
Bacteria—millions per c.c.
Ammonia—milligrams per c.c.
800
700
600
500
400
300
200
100
0·40
0·35
0·30
0·25
0·20
0·15
0·10
0·05
1
2
3
Weeks

Fig. 3.

the ammonia produced in the first week was higher in the "aerated" than in the "non-aerated" cultures. The relations between bacterial numbers and production of ammonia are, however, substantially the same in the "aerated" as in the "non-aerated" cultures, and they are therefore considered together in the statement of these relations.

When the cultures are all considered together, several interesting results emerge, which will now be discussed. The first of these is the *effect of diluting the inoculum*; this has been described for Cultures 7 and 8, and the same effect was observed in every case.

Table I.

Effect of diluting the inoculum.

Undiluted				Diluted			
Culture	Inoculum (millions)	Average bacterial nos. in first week (millions per c.c.)	Ammonia produced in first week (mgm. per c.c.)	Culture	Inoculum (millions)	Average bacterial nos. in first week (millions per c.c.)	Ammonia produced in first week (mgm. per c.c.)
1	1060	106	0·0236	2	132	22	Nil
3	1633	101	0·0247	4	158	21	Nil
5	1024	96	0·0316	6	94	20	Nil
7	1388	114	0·0406	8	131	23	Nil
9	1438	44	0·0140	10	162	25	Nil
11*	1100	316	0·1093	12*	103	108	0·0307
13*	1488	210	0·070	14*	196	72	0·0245
15*	1500	321	0·1170	16*	296	22	0·0019

* "Aerated" cultures.

It will be seen from Table I that both the bacterial numbers and the ammonia production are very much depressed during the first week by diluting the inoculum, and that this depression is apparent in the "aerated" as well as in the "non-aerated" cultures. In the "non-aerated" cultures no ammonia at all is produced in the diluted inoculum cultures during the first week, and the average bacterial numbers do not rise above 25 millions per c.c. The average ammonia production in the first week is 0·0047 mgm. per c.c. for all the "diluted" cultures, as against 0·054 mgm. per c.c. for all the "undiluted." Once the initial depression is over, however, the "diluted" cultures follow the same course as the "undiluted." Thus the average production of ammonia in four weeks after the reaction begins in Cultures 6, 8 and 10 is 0·233 mgm. per c.c., which compares well with the average of 0·292 mgm. per c.c. produced in Cultures 5, 7 and 9.

The next question to be considered is the *nature of the curve of ammonia production.*

As will be seen by reference to Figs. 1 and 2, the general form of the curve representing the amount of ammonia present is very similar to the curve of an autocatalytic reaction (14, 15). To test the correspondence of these curves with the autocatalytic curve, the relative rate of increase per cent. during any given week was plotted against the amount of ammonia present at the beginning of that week; in the case of an autocatalytic reaction the resulting graph should be a straight line (8). The results obtained from some of the cultures are shown in Fig. 4.

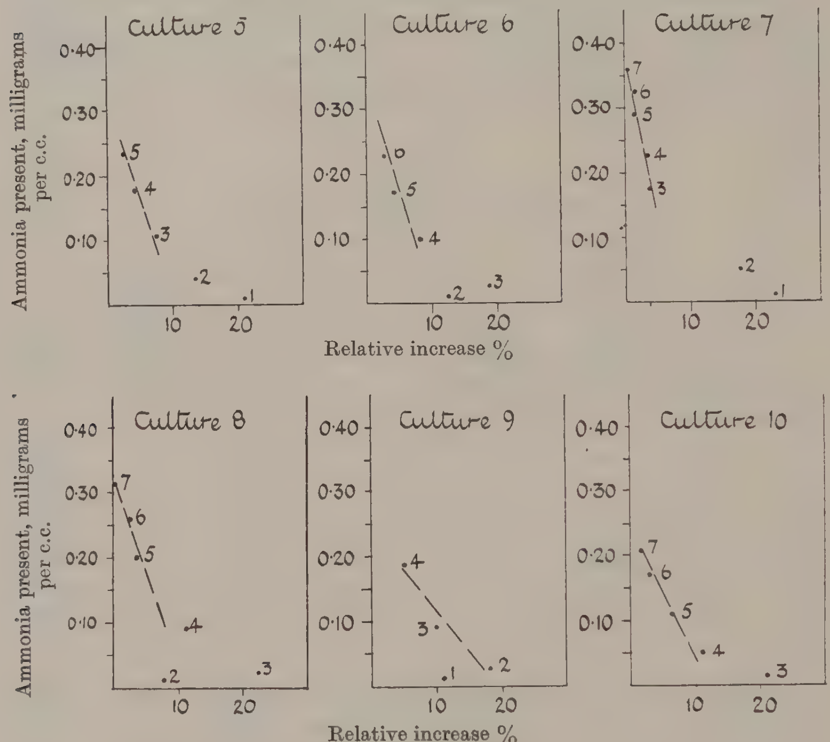


Fig. 4.

It will be seen that there is a very close approximation to a straight line in all cases, except for the readings obtained in the first week or two weeks, which deviate more or less from the straight line. This indicates that the curves of ammonia production obtained correspond to an autocatalytic curve only in the latter part of their course. There is evidently

some irregularity in the first part of the curve; this is also shown by the fact that in four cases (Cultures 7, 8, 15 and 16) the period of greatest increase does not coincide with the formation of half the theoretical amount of ammonia. Thus, in the case of Culture 7, the theoretical end-point of the reaction is reached at a concentration of about 0·4 mgm. ammonia per c.c.; half this amount, 0·2 mgm. per c.c., is present in the culture at some time during the third week (see Fig. 1); but the greatest reaction velocity is observed during the second week, when 0·126 mgm. ammonia is formed, as against 0·052 mgm. in the third week.

Change in reaction of the medium.

The reaction of the medium became more alkaline during the course of the experiment; as has been previously stated, the initial pH was 7·2 in every case, while the pH in those cultures which were kept for the full period of seven weeks was 8·5 at the end of the experiment.

RELATION BETWEEN BACTERIAL NUMBERS AND AMMONIA PRODUCTION.

In comparing bacterial numbers with ammonia production, it is first of all necessary to find a definite measure of each of these two variables to use as a basis for comparison. In the comparison which follows (Table II), the bacterial numbers have been measured as the average bacterial numbers per week in millions per c.c. (referred to as n), and the production of ammonia is stated as the increase in concentration of ammonia per 24 hours, in mgm. NH_3 per c.c. (referred to as P).

Now it will be plain from a study of Table II that the relation between these two quantities, P and n, is not one of simple proportion. If we consider Culture 7, for instance, we see that the greatest increase in concentration of ammonia occurs in the second week, when the average number of bacteria is about 450 millions per c.c.; the rate of increase then falls off in the third and fourth weeks, in spite of the fact that the numbers of bacteria are rising to an average of 670 millions per c.c. in the third week, and 890 millions per c.c. in the fourth week. On the other hand, a greater value of P in the second week compared to the first week is accompanied by a rise in average bacterial numbers, as is also the case in the third and fourth weeks of Culture 8.

If the rate of production of ammonia (P) were simply proportional to the average bacterial numbers (n), then the "efficiency" of the bacteria, *i.e.* the amount of ammonia produced by each individual organism, would be a constant quantity. As this is not the case, the

efficiency must be a variable quantity, depending on the values of P and n.

Table II.

Values of P *and* n.

Culture	Week after inoculation	n (millions per c.c.)	P (mgm. per c.c.)	Q
1	1	106	0·0034	0·0319
	2	241	0·006	0·0250
2	1	22	Nil	—
3	1	101	0·0035	0·0349
	2	227	0·0068	0·0301
	3	158	0·0081	0·0512
4	1	21	Nil	—
	2	70	0·0036	0·0522
5	1	96	0·0045	0·0464
	2	299	0·0095	0·0316
	3	161	0·0105	0·0649
	4	214	0·0081	0·0380
	5	230	0·0063	0·0276
6	1	20	Nil	—
	2	54	0·0022	0·0410
	3	336	0·0105	0·0312
	4	677	0·0106	0·0156
	5	660	0·0082	0·0124
	6	609	0·0073	0·0119
7	1	114	0·0058	0·0509
	2	448	0·0179	0·0401
	3	669	0·0074	0·0111
	4	890	0·0089	0·0100
	5	847	0·0048	0·0057
	6	901	0·0058	0·0064
	7	898	0·0023	0·0061
8	1	23	Nil	—
	2	27	0·0011	0·0416
	3	244	0·0104	0·0426
	4	705	0·0159	0·0225
	5	646	0·0082	0·0126
	6	556	0·0079	0·0143
	7	472	0·0008	0·0016
9	1	44	0·002	0·0458
	2	233	0·0094	0·0402
	3	536	0·0137	0·0202
	4	680	0·0116	0·0170
10	1	25	Nil	—
	2	22	Nil	—
	3	112	0·0054	0·0486
	4	320	0·0083	0·0260
	5	273	0·009	0·0329
	6	335	0·0053	0·0158
	7	361	0·004	0·0110
11	1	316	0·0156	0·0494
	2	512	0·0267	0·0522
	3	419	0·0126	0·0300
12	1	108	0·0044	0·0408

Table II (*continued*).

Culture	Week after inoculation	n (millions per c.c.)	P (mgm. per c.c.)	Q
13	1	210	0·010	0·0477
	2	443	0·021	0·0473
	3	210	0·013	0·0621
14	1	72	0·0035	0·0484
15	1	321	0·0167	0·0521
	2	524	0·0146	0·0279
	3	316	0·0096	0·0306
	4	177	0·0108	0·0609
16	1	22	0·0003	0·0122
	2	151	0·0101	0·0668
	3	284	0·0128	0·0453
	4	239	0·0083	0·0348

The efficiency was expressed as a value Q, such that

$$Q = P \times \frac{1000}{n},$$

i.e. Q is equal to the amount of ammonia that would be formed in 24 hours by 1000 million bacteria, under the conditions of the experiment. The value of Q was determined from the known values of P and n in every case except those where P was zero, *i.e.* where there was no increase in concentration of ammonia during the week in question. These values are given in the table with the values of P and n.

In investigating the relation of Q to n, the first question to be answered is: does Q increase or decrease in value as n increases? If the numbers of bacteria are grouped in hundreds of millions, the average values of Q for each group are as given in Table III. It will be seen that, on the whole, the value of Q diminishes as the value of n increases.

Table III.

Average bacterial numbers (millions)	No. of cases	Average value of Q	Average bacterial numbers (millions)	No. of cases	Average value of Q
Over 800	4	0·00706	300–400	7	0·0309
700–800	1	0·0225	200–300	12	0·0382
600–700	6	0·0134	100–200	9	0·0501
500–600	4	0·0287	Under 100	7	0·0411
400–500	4	0·0298			

If the values of Q are grouped in 0·005 mgm., and the values of n in hundreds of millions, the numbers of cases in each group are shown by the following correlation table (Table IV).

Table IV.

	Under 100	100–	200–	300–	400–	500–	600–	700–	800–	900–	No. of cases
Q under 0·005	.	.	.	.	1	.	.	.	.	.	1
0·005–	.	.	.		.	.	.	.	2	1	3
0·01–	1	.	.	1	.	1	4	.	1	.	8
0·015–	.	.	.	1	.	.	2	.	.	.	3
0·02–	1	.	.	.	.	1	.	1	.	.	3
0·025–	.	.	2	1	.	1	.	.	.	.	4
0·03–	.	2	2·5	2·5	1	.	.	.	.	.	8
0·035–	.	.	1	.	.	.	.	.	.	.	1
0·04–	2	1	2	.	1	.	.	.	.	.	6
0·045–	2	1	2	1	1	.	.	.	.	.	7
0·05–	1	2	.	1	.	1	.	.	.	.	5
0·055–	.	.	1	.	.	.	.	.	.	.	1
0·06–	.	2	1	.	.	.	.	.	.	.	3
0·065–	.	1	.	.	.	.	.	.	.	.	1
No. of cases	7	9	11·5	7·5	4	4	6	1	3	1	Total 54

The form of this correlation table at once suggests a linear relationship between Q and n; that is, that Q falls off regularly, from some fixed value for small values of n, to zero as n approaches 1000.

The ungrouped data were examined statistically with the following results:

Mean value of $Q = 0{\cdot}0329$, if $n = 350$. Regression efficient of Q on $n = -0{\cdot}00004857$. Therefore

$$Q \times 10^4 = 499 - 0{\cdot}486\, n.$$

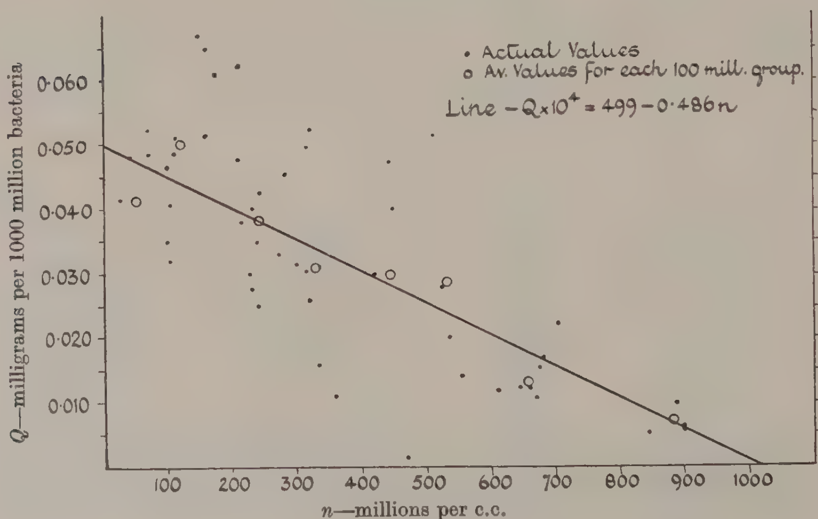


Fig. 5.

This relationship indicates that Q should have a value about 0·05 when n is zero, and should thereafter fall off regularly until it vanishes altogether about $n = 1000$. There is no question of the significance of this relationship; the standard error of the regression coefficient is 0·0000070, showing that it differs significantly from zero.

Since the relation between Q and n is linear, another way of expressing it is to say that the correlation coefficient between Q and n is $-0{\cdot}695$; and again, as may be seen by consulting R. A. Fisher's table of significance levels of the correlation (8), as there are 52 degrees of freedom, there is no question of the significance of a correlation coefficient of this magnitude.

Having obtained a definite negative linear relation between Q and n, the question that next arises is: in what measure is this high value of the correlation coefficient due to the effect of time on the two variables? That is, is the relation between Q and n an illusory one, due simply to the fact that n increases with time while Q decreases?

If the observations of n and Q are grouped in weeks, the average values as shown in Table V are obtained:

Table V.

Week after inoculation	Mean value of n	Mean value of Q	No. of cultures
1	137·3	0·04186	11
2	269·1	0·04133	12
3	313·2	0·03980	11
4	487·8	0·02810	8
5	531·2	0·01824	5
6	600·2	0·01210	4
7	577·0	0·00623	3
	Mean 349·5	0·03291	Total 54

It can be clearly seen from this table that the average value of n rises in successive weeks, whereas the average value of Q decreases with time. It is, therefore, necessary to discover whether the relation already stated to hold between Q and n is entirely due to the time factor, or if there is a definite relation between Q and n independent of time.

If the observations are grouped in weeks, and the variations of Q and n within each week considered separately from their variations from one week to another, the following results are obtained (Table VI):

Table VI.

	Degrees of freedom	Regression of ($Q \times 10^4$) on n	Correlation coefficient
Between weeks	6	−0·6821	−0·9093
Within weeks	47	−0·3499 (significant)	−0·5295 (significant)
Total	53	−0·4857	−0·6951

This shows that while there is a large negative association between Q and n due merely to the time factor, yet there is also a significant negative association which is independent of time. The old regression figure of $-0{\cdot}486$ becomes $-0{\cdot}350$ independent of time, and the correlation coefficient becomes $-0{\cdot}5295$, but these values are still strongly significant.

The relation between Q and n, independent of time, can now be expressed in the form

$$Q \times 10^4 = 451{\cdot}4 - 0{\cdot}350n.$$

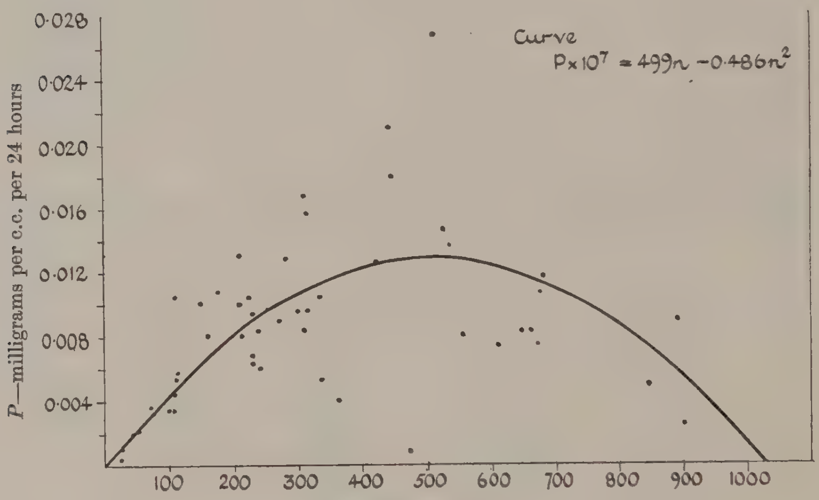


Fig. 6.

Now it was stated earlier that the relation between P and n was not one of simple proportion, so that it could not be expressed in terms of a linear relation, or in terms of a correlation coefficient. Any theoretical difficulties in the way of determining this relationship have, however, been avoided by the calculation of the quantity Q, which bears a simple relation to P, and which exhibits so strongly a linear relation to n.

For, as $Q = \dfrac{P \times 1000}{n}$, the regression equation for Q can be transformed into an equation in terms of P.

From the equation of the total regression of Q on n, including the effect due to time, we therefore obtain the following equation in terms of P:

$$P \times 10^7 = 499n - 0{\cdot}486n^2.$$

This is a parabolic relationship, and indicates a zero value for P not only when $n = 0$, but also when n is of the order of 1000. P will attain a maximum at a point determined by differentiating the above equation:

$$499 - 2 \times 0{\cdot}486n = 0.$$

Therefore $$n = \frac{499}{0{\cdot}972} = 513.$$

So that for n approximately 500, P attains a maximum value of about 0·0128. The curve shows the fifty-four observed points and the parabola fitted to these by means of the relations given.

Similarly, from the relation found between Q and n, independent of time, an equation in terms of P can be obtained which is also independent of time:

$$P \times 10^7 = 451{\cdot}4n - 0{\cdot}350n^2.$$

In this case P will have a zero value when $n = 0$, and again when $n = 1290$, and will attain its maximum value when $n = 645$. Apart from the effect of time on P and n, the relation between these two variables is still a parabolic one.

II. SAND CULTURES.

METHODS.

Pure cultures of the soil bacterium "YB" were used, either alone or with pure cultures of the amoeba *Hartmanella hyalina*, grown with "YB."

These organisms were grown in sand moistened with a food solution. The sand was washed with hydrochloric acid and then with water, and ignited; the required amount (400 or 500 gm.) was then autoclaved for 15 minutes at 15 lb. pressure in a 2 litre flask.

The solution used was a 0·5 per cent. solution of peptone (B.D.H.) in a salt solution of the following composition: NaCl 0·06 per cent., KCl 0·001 per cent., $CaCl_2$ 0·002 per cent., $MgSO_4$ 0·001 per cent.

The pH of the solution was adjusted to 7·3 with $N/10$ sodium hydroxide (except in experiment VII, where 0·5 per cent. peptone in soil extract was used). The total nitrogen content of the peptone solution was 0·74 mgm. per c.c.

Suspensions, either of "YB" alone or together with amoebae, were made in the food solution, and the sand was then inoculated with these suspensions by means of a sterile pipette. 1 c.c. of liquid suspension was added for every 10 gm. of sand, except in the first experiment, where 50 c.c. of liquid was used with 400 gm. of sand.

Except in the first experiment, where the only aeration was through a bent glass tube inserted in the cork of the flask, a slow stream of air was drawn over the cultures by an aspirator. The outgoing air was passed through an acid trap to recover any ammonia carried over.

The bacteria and amoebae were counted by the methods in use in this laboratory (1); in the last experiment of the series, however, the amoebae were not counted, but their presence or absence was determined.

Ammonia was determined by the method of Harper (9), a sample of 25 gm. being used for each determination. The standard sodium hydroxide used for titration had a normality of 0·15–0·17 N. Owing to the size of the sample required, it was not possible to make duplicate determinations.

In four of the experiments recorded, I, III, VII and VIII, the bacteria were counted daily; in the other experiments, which were run for longer periods, the bacteria were only counted when the ammonia determinations were made.

The amoebae were counted daily in experiments I and III, and when the ammonia determinations were made in the other experiments.

The methods adopted in each of the experiments recorded here are given in Table VII.

Table VII.

Details of methods in sand experiments.

Culture	Amoebae	Length of experiment (days)	Days when ammonia was determined	Amoebae alive till	Bacteria counted (days)
I. A	Present	12	4, 8, 12	End of exp.	Daily
B	Absent	12	4, 8, 12		,,
III. A	Present	12	7, 12	10th day	Daily
B	Absent	12	7, 12		,,
IV. A	Present	31	1, 13, 20, 31	End of exp.	1, 13, 20, 31
B	Absent	31	1, 13, 20, 31		1, 13, 20, 31
VI. A	Present	46	1, 7, 20, 32, 39, 46	End of exp.	1, 7, 20, 32, 39, 46
B	Absent	46	1, 7, 20, 32, 39, 46		1, 7, 20, 32, 39, 46
VII. A	Present	20	8, 12, 16, 20	End of exp.	Daily
B	Absent	20	8, 12, 16, 20		,,
VIII. A	Present	28	7, 14, 21, 28	21st day	Daily
B	Absent	28	7, 14, 21, 28		,,

Results.

The results obtained from the sand cultures are given in Table VIII.

For purposes of comparison with the liquid cultures described in the second part of this paper, the results are given in terms of c.c. of the food solution.

The bacteria are recorded in millions per c.c. and the ammonia as mgm. NH_3 per c.c.

Table VIII.

Results of sand experiments.

Culture	Days after inoculation	Average bacterial nos. (millions per c.c.)	Average no. of amoebae (per c.c.)	Ammonia present at end of period (mgm. per c.c.)	Increase in ammonia per 24 hr. (mgm. per c.c.)
I. A	0–4	5,791	8,300	0·119	0·030
	4–8	8,391	12,276	0·184	0·016
	8–12	11,056	70,000	0·288	0·026
B	0–4	9,321	None	0·144	0·036
	4–8	11,190	,,	0·209	0·016
	8–12	6,486	,,	0·263	0·017
III. A	1–7	2,228	30,410	0·282	0·030
	7–12	312	26,650	0·341	0·012
B	1–7	4,221	None	0·295	0·037
	7–12	2,358	,,	0·326	0·006
		Bacteria present (millions per c.c.)	Amoebae present (per c.c.)		
IV. A	0	36	—	—	—
	1	36,500	275	0·123	0·123
	13	760	58,300	0·326	0·017
	20	1,078	40,700	0·299	−0·004
	31	369	75	0·299	0
B	0	32	None	—	—
	1	12,546	,,	0·094	0·094
	13	24,745	,,	0·286	0·016
	20	1,040	,,	0·312	0·004
	31	44	,,	0·361	0·004
VI. A	1	435	275	0·070	0·070
	7	1,144	396,000	0·158	0·015
	20	765	1,190,200	0·356	0·015
	32	28	367,400	0·519	0·014
	39	110	19,800	0·475	−0·006
	46	44	165	0·321	−0·022
B	1	671	None	0·070	0·070
	7	935	,,	0·141	0·012
	20	374	,,	0·321	0·014
	32	88	,,	0·510	0·016
	39	66	,,	0·392	−0·017
	46	132	,,	0·356	−0·005
		Average no. of bacteria (millions per c.c.)			
VII. A	0–18	1,599	40,700 (6 days)	0·387	0·048
	8–12	534	7,040 (12 ,,)	0·466	0·020
	12–16	795	—	0·519	0·013
	16–20	414	418 (20 ,,)	0·541	0·006
B	0–8	1,227	None	0·246	0·031
	8–12	1,379	,,	0·299	0·013
	12–16	2,335	,,	0·312	0·003
	16–20	2,574	,,	0·528	0·054
VIII. A	1–7	5,469	Present	0·331	0·043
	7–14	949	,,	0·550	0·031
	14–21	414	,,	0·568	0·003
	21–28	409	—	0·515	−0·008
B	1–7	5,322	None	0·317	0·041
	7–14	3,280	,,	0·405	0·013
	14–21	1,003	,,	0·405	0
	21–28	503	,,	0·396	−0·001

Though these results are neither sufficiently numerous nor sufficiently accurate for detailed statistical analysis, yet there are one or two points of interest that arise from a comparison of these sand cultures with the liquid cultures (see Part I), and also from a comparison of the cultures containing amoebae with those without amoebae.

If we compare the sand cultures as a whole with the liquid cultures, we see first of all that the numbers of bacteria are very much higher in sand; in experiment IV counts of 36,500 million and 24,745 million per c.c. were recorded, and average counts of several thousand million per c.c. are common; whereas in the liquid cultures the highest actual count was 1190 million per c.c., and the highest average count 901 millions per c.c.

The rate of increase in concentration of ammonia is also higher in the sand cultures, reaching a maximum value of 0·123 mgm. of ammonia in 24 hours, as against the maximum figure of 0·0267 mgm. in 24 hours obtained in liquid cultures. Following on this greater rate of production of ammonia, the whole reaction is seen to proceed much more quickly in sand cultures. In the sand cultures the ammonia content rises to a maximum after three or four weeks, and then some loss of ammonia is observed (this ammonia was recovered in the acid traps); in the liquid cultures the ammonia content was still increasing at the end of seven weeks, and in no case was any loss of ammonia observed.

The bacteria also seem to be able to decompose a larger proportion of the peptone in sand cultures, judging by the percentage of the total nitrogen in the medium that is obtained as ammonia.

In the liquid cultures the concentration of ammonia at the end-point of the curve of ammonia production corresponds to only 47·5 per cent. of the total nitrogen.

On the other hand, in the sand cultures, the highest concentrations of ammonia obtained, expressed as percentages of the total nitrogen, are as shown in Table IX.

Table IX.

Culture		Days after inoculation	% of total nitrogen as ammonia
VI.	A	32	58
	B	32	57
VII.	A	20	60
	B	20	59
VIII.	A	21	63
	B	14	45

In comparing the cultures containing amoebae with those containing bacteria alone, since the effect of the presence of the amoebae on ammonia production is the principal point to be considered, it is during the period when the concentration of ammonia is rising (that is, the first three weeks after inoculation) that this comparison would be profitable.

If we compare each period between ammonia estimations in every culture containing amoebae with the same period in the corresponding culture containing only bacteria, we obtain the following results: During the first three weeks after inoculation, in twelve cases out of seventeen the bacterial numbers are *lower* over a given period in a culture containing amoebae than in its corresponding culture containing bacteria alone. But in thirteen cases out of seventeen the rate of production of ammonia is *higher* in the cultures containing amoebae, and in one case the rate of ammonia production is equal in the two corresponding cultures.

These results can also be expressed in terms of percentages as follows: in the first three weeks after inoculation the presence of amoebae causes a depression in the bacterial numbers in 70 per cent. of cases, but the cultures containing amoebae have a higher rate of ammonia production in 76 per cent. of cases.

DISCUSSION.

On considering the results reported in the first half of this paper, it is evident that, though the course of the reaction (or series of reactions) which produces ammonia does not correspond exactly to the course of bacterial growth, yet there is a definite relation between the two quantities. As the bacterial numbers increase, the rate of production of ammonia increases too until a maximum level is reached, and then with any further increase in bacterial numbers the rate of ammonia production decreases. It must be remembered, however, that there appears to be a minimum bacterial content (somewhere about 25 millions per c.c.) below which no ammonia appears to be formed at all. Now this parabolic relation between bacterial numbers and rate of ammonia production has been shown to be partly due to time. That is, that the bacterial numbers on the whole tend to rise continuously throughout the course of the experiment, while the rate of production of ammonia rises to a maximum and then falls off. But it has also been shown that there is a relation independent of time between bacterial numbers and rate of ammonia production, which is still of a parabolic nature.

If we attempt to find an explanation for the nature of this relation, there appear to be several possibilities. The first is that ammonia is not

really an end-product of the reaction whereby the bacteria are obtaining their energy; that is to say, the constituent amino-acids of the peptone are first de-aminised, and that the bacteria then utilise the residue of the molecule as a source of energy. This view of the decomposition of proteins by bacteria was put forward by Butkevitch (quoted by Waksman and Lomanitz)(21). According to this view, the rate at which ammonia was formed would not be an index of bacterial activity. This would explain the continued growth of bacteria after the rate of formation of ammonia had begun to decrease.

This explanation, however, seems to me to be insufficient for the following reasons: the same kind of relationship between efficiency (Q) and bacterial numbers that has been found in these experiments to obtain for ammonia formation, has also been observed by Cutler and Crump(5) in the case of the formation of carbon dioxide by "YB." In this case, also, there is an inverse relation between efficiency and bacterial numbers.

Now in the case of carbon dioxide there is no doubt of its being an end-product of the energy reactions of bacteria; and since the relation between bacterial numbers and efficiency is of the same kind in both cases, it seems as if ammonia can also be regarded as an end-product of the reaction from which the bacteria are obtaining their energy. It follows, that the rate of ammonia production will be an index of the activity of the bacteria, and that this activity, therefore, reaches a maximum at an intermediate value of bacterial numbers, falling off both when the numbers increase above this intermediate value, and when they fall below it. It is difficult to account for this falling off in activity with rising bacterial numbers above the "optimum" value; the results obtained do not in themselves indicate any cause for this, though several conjectures might be made. For instance, the production of a de-aminising enzyme by the bacteria may be inhibited by the presence of too great a number of bacteria.

When we come to consider the results recorded in Part II of this paper, some light may be thrown on them by the experiments already discussed, although it should be remembered that the two sets of results are not strictly comparable. The differences between the results obtained in sand and in liquid cultures have already been pointed out; a similar increase in ammonia production and bacterial numbers in sand or soil cultures as compared with liquid cultures was observed by Löhnis and Green(12, 13), and was attributed by them to the greater degree of aeration obtained in sand or soil cultures.

Now the results obtained from a comparison of the cultures containing amoebae with those containing bacteria alone were of this nature; the presence of the amoebae, while they lower the bacterial numbers, yet gives an increase in the rate of ammonia production. Now, unless we adopt the view that the amoebae themselves are capable of decomposing the peptone, and so increasing the ammonia produced, the explanation of this raising of the rate of ammonia production while the bacterial numbers are lowered seems to me to lie in the results already discussed. That is to say, that the amoebae are reducing the bacterial numbers from too high a value to one nearer the "optimum" value for bacterial activity, and so are automatically increasing the rate of ammonia production.

It must be observed that it is not suggested that the presence of amoebae will increase the amount of ammonia finally formed; what they do increase is the rate at which it is formed. It has been shown in Part I that the latter part of the course of the reaction in liquid cultures corresponds to an autocatalytic reaction, that is to say, the reaction is retarded by the accumulation of its own products. The effect of increasing the rate of ammonia formation will therefore be, not to increase the amount of ammonia formed at the end of the reaction but simply to carry the reaction through more quickly, provided that, as in the experiments recorded here, the products of the reaction are allowed to accumulate. In the sort of conditions actually obtaining in the soil, however, the products of such a reaction would be removed by further bacterial action as they were produced, and under these conditions the presence of protozoa, by increasing the speed of the formation of ammonia, might also increase the amount eventually formed.

SUMMARY.

1. Two sets of experiments on ammonia production from peptone were carried out, one with "YB" bacteria in liquid cultures, and another comparing "YB" and *Hartmanella* with "YB" alone in sand cultures.

2. In liquid cultures an inverse linear relation was found to hold between bacterial numbers and efficiency.

3. The greatest rate of production of ammonia was found to correspond to a bacterial content of about 500 million per c.c., any increase in bacterial numbers above this lowered the rate of ammonia production.

4. The effect of diluting the inoculum ten times was to increase the "lag" period, both for bacterial growth and for ammonia production,

to a week or more; after this extended "lag" period the cultures then followed a normal course.

5. In sand cultures the presence of amoebae, while lowering the bacterial numbers, appears to increase the rate of ammonia production.

6. It is suggested that the amoebae reduce the bacterial numbers from too high a value to a value nearer the optimum for ammonia production, and so increase the rate of ammonia production.

I should like to express my thanks to Mr Woolf, of the Sir William Dunn Institute of Biochemistry, Cambridge, for demonstrating the details of his method to me, and also to Dr Wishart and Dr Fisher, of the Statistical Department, Rothamsted, for help in the analysis of the results, and especially to Mr D. Ward Cutler, in whose department the work recorded in this paper was carried out, for his constant help and encouragement.

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ON THE RESISTANCE OF BASKET WILLOWS TO BUTTON GALL FORMATION

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Introduction.

Of the three gall midges (Cecidomyidae) which commonly do damage to basket willows (namely, *Rhabdophaga heterobia* H.Lw., *R. terminalis* H.Lw. and *R. saliciperda* Duf.), *R. heterobia* is the most important, while *R. terminalis* occasionally occurs in epidemic numbers. *R. heterobia*[1] is the common midge whose larvae produce either button-like galls on the terminal growing points, swollen bud galls on the lateral over-wintering buds, or swollen catkin galls. Of these the button gall is the most serious, as there follows a very great decrease in end growth, while the production of side shoots from the lower lateral buds is stimulated. It was thought advisable to determine whether different varieties and species of willow showed varying degrees of resistance to attack. The facilities given by the Agricultural and Horticultural Research Station, Long Ashton, enabled the writer to make observations on the Long Ashton variety beds, and also to set up trials at Rothamsted. This note is in the nature of a preliminary report of the work which is being continued. It is hoped to test out all the most important varieties in order to gain information as to the most resistant kinds of basket willow.

Results of examination of Long Ashton trial beds.

In order to obtain some idea of varietal resistance it was decided to make annual observations on the willow variety beds. In 1927 and 1928, nineteen varieties of *S. triandra* and the hybrid *viminalis* × *triandra*[2], known as Black Top, were examined. In both years it was obvious from the figures obtained that some varieties were very liable to attack while others were less so. In 1929 the infestation by the midge was so slight that it was considered useless to try to differentiate between the attacks on the different varieties. It was found that some varieties which were estimated to be free from attack in 1927 were attacked in 1928. This was possibly due to the fact, discovered later, that the oviposition of eggs in

[1] Barnes, H. F., "Button Top" of basket willows, *Journ. Min. Agric.* April 1929, pp. 65–71.

[2] Botanically named *hippophaefolia*.

the terminal bud by the midge does not always result in the formation of a button gall: sometimes the shoot merely dies off, and in such a case as this it would be extremely probable that the attack would be overlooked.

Rothamsted variety trials, 1929.

In order to obtain more critical results it was decided to set up variety trials at Rothamsted under controlled conditions. A glasshouse which had four beds 12 × 3 ft. was used and each bed, after being planted with sets of different varieties, was covered with a muslin cage 12 × 3 × 6 ft. high suspended from the roof and with its canvas base dug into the soil. This prevented insects from either entering or leaving the cages and plants. On each side of the cage two sleeves were made in order to insert button top midges. The roof of the house was partially whitewashed to keep off the sun's rays during the hottest part of the day; all the windows and doors were left open and the floor was flooded periodically to ensure sufficient moisture being present.

The varieties of basket willow were randomised, four to six sets of each variety being planted in each bed in such a way that midges had equal chances of alighting on any of the four or three varieties present. In the first cage there were four varieties—Champion, Black Maul, Newkind and Harrison, the latter a hybrid *purpurea* × *viminalis*, and in the second cage three varieties—Light French, Stone Rod and Pomeranian. The third cage had three varieties, but the experiment was spoilt by an attack of the aphid, *Chaitophorus capreae* Koch., whose eggs had undoubtedly been accidentally introduced on the sets.

Given numbers (16 and 20) of female midges, which had previously been observed to have been impregnated, were put into the cages through the sleeves on May 26th and 27th. No further additions were made, but those present were left to multiply unchecked till the end of the season. Three broods of adults attacked the willows, those inserted in May and two generations of their descendants, the F_1 about July 11th–15th, and the F_2 about August 19th. On June 21st all the galls of the parent midges were marked and counted, and on August 9th all the galls of their first generation were likewise marked and counted. The galls of their second generation were not counted, as it was obvious that very great midge overcrowding had taken place, and any count would not give any true idea of attack. It was observed, however, that the variety Harrison did not suffer attack by the second generation. The reason for putting in so few females at the beginning of the experiment was to make

the trial preferential at the beginning of the season and then less so in the next generation of midges and obligatory in the second generation of descendants of the original midges.

The results of the counts are shown in the following table, where it will be seen that the Harrison variety has so far been immune.

Variety	No. of shoots available to		No. of button galls produced by		No. of shoots killed but without gall formation by		% attack by	
	Parent midges	F_1 midges	Parent midges	F_1 midges	Parent midges	F_1 midges	Parent midges	F_1 midges
Bed 1								
Champion	24	117	13	77	3	10	66	74
Black Maul	17	39	8	26	1	5	53	80
Newkind	21	122	17	76	2	25	86	83
Harrison	18	18	0	0	0	0	0	0
Bed 2								
Light French	27	221	0	5	16	83	59	40
Stone Rod	22	89	12	67	7	12	86	89
Pomeranian	32	138	10	80	2	36	38	84

From this table it will be seen that button galls develop almost whenever eggs are laid in some varieties, in others, *e.g.* Light French, quite a considerable number of shoots die without forming such galls. Although the same result follows, *i.e.* side branching, it is valuable to have such varieties as the midges do not develop and increase to the same extent as if every attacked shoot developed a button gall. The extent of side branching can also be seen from the table; for example, Champion started with 24 shoots, but after one attack had 117 shoots.

Conclusions.

Different varieties of basket willow show different susceptibility to attack by the button-top midge.

Whereas one of the varieties tested, Harrison, showed a complete immunity through three generations of midges, the remaining five varieties were heavily attacked.

The shoots of some varieties, although attacked, do not always form button galls, nevertheless side branching occurs.

Acknowledgments.

I have to express my indebtedness to Mr H. P. Hutchinson for much advice and for supplying me with willow sets, and also to Dr C. L. Walton and Mr L. N. Staniland for assistance in estimating the attacks on the Long Ashton variety beds.

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PROCEEDINGS OF THE ASSOCIATION OF ECONOMIC BIOLOGISTS. I

Ordinary Meeting held at 2.30 p.m. on Friday, February 21st, 1930, in the Botanical Lecture Theatre of the Imperial College of Science and Technology, London. The Chair was taken by the President, Dr A. D. Imms, F.R.S.

THE UTILITY OF MATHEMATICAL METHODS IN RELATION TO WORK ON BIOLOGICAL CONTROL.

By W. R. THOMPSON, Ph.D., D.Sc.
(*Imperial Bureau of Entomology.*)

The term "biological control" may be used to designate either the check on the multiplication of plants and animals exerted by other organisms, or the methods of economic entomology by which we seek to reduce or prevent loss through noxious plants and insects by the employment of other and beneficial organisms which prey upon them in various ways. It is in the latter sense that the term is used in this address.

Although work on biological control has been going on for a considerable number of years, our knowledge in regard to this subject has not advanced very rapidly. The reason for this is that the process of biological control presents peculiar difficulties as compared, for example, with the methods of mechanical control, such as spraying, which can be conducted on orthodox experimental lines. In the most common form of experiments on biological control we introduce the beneficial insect attacking a noxious animal or plant into an environment in which it has never before been observed, and which is positively known to resemble its original environment only in the presence of the host organism. It is, however, obvious that the effectiveness of the introduced parasite or predator will depend not simply on the presence and abundance of the host but on a precise and definite combination of all the environmental factors. If we introduce a beneficial insect into two different regions inhabited by the same host insect, the results may be very different. For example, according to Tillyard, *Aphelinus mali* in New Zealand is very effective as a control for the Woolly Aphis, whereas in England and on the Continent this parasite seems to be rather unimportant. Similarly, although *Novius cardinalis* has proved an effective remedy for the Fluted Scale in practically all parts of the world, I am informed by Dr Bodenheimer that it is not always a perfect control for this pest in Palestine. The introduction of a beneficial insect into new countries thus constitutes, in a certain sense, a new and unrepeatable experiment. It is, therefore, very hard to draw any conclusions of general interest from the past and present work on biological control, especially since it is often a very long time before any experiment can be considered as concluded. This makes it very difficult to conduct this work on anything but purely empirical lines, though all entomologists will agree that the organisation of the work on a scientific or rational basis is highly desirable.

Now here, as elsewhere in natural science, the difficulty arises chiefly from the bewildering perplexity of events as they occur in nature. It would be impossible to follow, even during the course of a single season, the complicated interactions between an organism, its parasites and predators, and the various environmental factors. The only remedy for this difficulty is to attempt to simplify the problem, which may be defined for the purpose of the present argument as the problem of the interactions of noxious animals and plants and their insect parasites or predators.

There are two ways in which this simplification may be attempted: the first is by experimental analysis, and the second by mathematical reasoning. The simplification produced by both of these methods is, of course, artificial, since in each case the complications which we find in nature are avoided by limiting ourselves to certain particular aspects of the problem. Each method, however, has its particular advantages and disadvantages.

The advantage of the experimental method is that it enables us to deal with the actual living organism, and thus provides us with information concerning the habits characteristic of certain definite species. Its disadvantage is that any conclusions that we draw from it are limited by the specific nature, or the specific characteristics of the organism we have used in our experiment. These results cannot necessarily be applied to natural phenomena in a general sense. The disadvantage of the mathematical method lies in the fact that we can obtain from it no information as to the course of events in any particular case. It will not give us any information about the characteristic behaviour of a parasite belonging to a particular species. Its advantage lies, however, in the fact that it deals simply with numbers, so that its conclusions have an extremely wide and, indeed, a universal application.

It is, of course, evident that questions relating to numbers or, in other words, questions of the quantitative order, are of primary importance in economic entomology. The problems the economic entomologist is asked to take up arise because the farmer finds that he is not getting a sufficient amount of money for his crop, having regard to the time he has spent in cultivating it, and the money he has invested in machinery and land. The damage from which he suffers depends, so far as its intensity is concerned, chiefly upon what we may call the number of individuals of the pest per unit of area. The number of individuals per unit of area depends chiefly, other things being equal, upon the original number of individuals of the pest in the field, the length of time they have been there, and their rate of increase during that time. Any entomologist who has the slightest experience with practical work in biological control realises that those problems are, to a very great extent, problems of a quantitative order. It seems, therefore, on general grounds that mathematical methods ought to be of considerable assistance in work of this type.

Broadly speaking, there are two principal ways in which mathematical methods can be applied to the numerical problem with which we meet in biology. The first is known as the method of *empirical formulae*, and the second as the method of *theoretical formulae.*

The method of *empirical formulae* is sometimes described as curve-fitting. Let us suppose that we have conducted a series of experiments to determine the relation between the length of life of an organism and the temperature of the environmental air. When the experiments are completed, we plot the values obtained in a system of rectangular co-ordinates of which the ordinates designate the temperature of the

environment in degrees Centigrade and the abscissae the life in days or hours. From these points we obtain a curve. We then seek among the various families of mathematical curves for one which is morphologically identical with the one corresponding to our experimental results, and when this curve, which of course corresponds to a definite mathematical formula, has been found, the problem may be considered for practical purposes as solved. This method has been employed in a vast number of physical and biological problems, and is certainly of great utility. It is, however, impossible to utilise it in problems of biological control, because we do not at present possess the necessary data.

The method of *theoretical formulae* is quite different. In this method we begin with a statement in ordinary language of the manner in which the quantitative or numerical values we are considering actually arise in nature. We then translate this statement into mathematical or symbolical language, after which we examine it and analyse it by the use of mathematical artifices of various kinds. In order to make clear the nature of the method a simple example may be taken. Let us suppose that we have twenty host insects, producing each two offspring per generation, and two parasites producing each three offspring per generation, and that each one of the progeny of the parasite destroys one of the progeny of the host. The course of events will then be as follows:

In Generation 1 we shall have (H = host, P = parasite):

$$\begin{aligned} H_1 &= 20 \times 2 = 40 \\ P_1 &= 2 \times 3 = 6 \\ & \overline{34} \text{ hosts remaining} \end{aligned}$$

Generation 2:

$$\begin{aligned} H_2 &= 34 \times 2 = 68 \\ P_2 &= 6 \times 3 = 18 \\ & \overline{50} \text{ hosts remaining} \end{aligned}$$

Generation 3:

$$\begin{aligned} H_3 &= 50 \times 2 = 100 \\ P_3 &= 18 \times 3 = 54 \\ & \overline{46} \text{ hosts remaining} \end{aligned}$$

Generation 4:

$$\begin{aligned} H_4 &= 46 \times 2 = 92 \\ P_4 &= 54 \times 3 = 162 \end{aligned}$$

Thus in the fourth generation the number of progeny of the parasite come to be superior to the number of the progeny of the host, which can then be considered as exterminated.

It is clear that this example simply describes in a very rough and general way the course of the interaction of a parasite and its host as it is actually observed in nature, and although it is only a very rough and general statement of the case, it does not contain anything inherently improbable.

On the other hand, it is evident that the example has certain limitations.

It has, in the first place, certain *explicit limitations*, that is to say, *it applies only to certain quantitative values explicitly designated*; ten hosts each producing two offspring, two parasites each producing three offspring. In the second place, the example has certain *implicit limitations*, which may not have been perceived at the time it was

being framed, but which, nevertheless, exist. Thus (1) the organisms are considered to be all parthenogenetic female-producing females; no males are allowed for in the example; (2) the successive generations are considered to be distinct and do not overlap; (3) the generations of the host and parasite are considered to be synchronous and all hosts available for attack by the parasite; (4) the distribution of both host and parasite is considered to be relatively homogeneous during the whole course of the process, or, if the distribution of one in relation to the other changes in any way this is not considered to alter the interaction of the two species in any significant way; (5) the parasites are considered to attack only unparasitised hosts; (6) the parasites are considered to constitute a homogeneous group, belonging, for example, all to a single species; (7) the phenomenon, and all the processes involved in it, is supposed to take the same course in every generation considered. These are only a few of the implicit limitations which we could mention. It is obvious, of course, that while they do not make such considerations as we may deduce from the study of the example absolutely valueless, they greatly restrict its significance. In order to extend this we must, therefore, attempt to find some way of removing these limitations.

The removal of the explicit limitations in the example can, of course, be effected in a very simple manner by the substitution of symbols for numbers. Thus, let the initial number of hosts in the example be represented by n, the initial number of parasites by p, the number of offspring produced by each individual of the host by h, and the number of offspring produced by each individual of the parasite by s. We may then proceed as follows:

Generation 1:

$$\begin{aligned} H_1 &= n \times h = nh, \\ P_1 &= p \times s = ps \\ &\overline{nh - ps} \text{ hosts remain.} \end{aligned}$$

Generation 2:

$$\begin{aligned} H_2 &= (nh - ps) \times h = nh^2 = psh, \\ P_2 &= \;ps \times s \qquad\quad = ps^2 \\ &\overline{nh^2 - psh - ps^2} \text{ hosts remain.......} \end{aligned}$$

Generation t:

$$H^t = nh^t - psh^{t-1} - ps^2h^{t-2} \qquad \text{to } t \text{ terms } ......(1).$$

$$P_t = ps^t \qquad ......(2).$$

The expression giving the number of hosts is obviously a geometrical series and can be summed, so that we finally obtain for the number of individuals of the host and parasite at the end of t generations the formula:

$$\left.\begin{aligned} H_t &= nh^t - ps\left\{\frac{s^{t-1} - h^{t-1}}{s - h}\right\} \\ P_t &= ps^t \end{aligned}\right\} \qquad ......(3).$$

This formula, though complicated in appearance, is, of course, nothing more than a symbolical, or shorthand representation of the simple chain of mathematical reasoning in the original numerical example.

It is very easy to show how the substitution of symbols for numbers has extended the significance of the formula. For instance, in our original example, the reproductive rate of the parasite was supposed to be different from that of the host, but because of the explicit limitations of the example, no definite conclusion could be drawn as to the

significance of this particular fact. Let us now suppose that the reproductive rate of the host be considered to be equal to the reproductive rate of the parasite; that is to say, let us put h equal to s. Our formulae then become:

$$H_t = nh^t - ph^t\,(t-1) \qquad \text{......(4).}$$
$$P_t = ph^t \qquad \text{......(5).}$$

Now let us suppose that, under these conditions, we wish to know how long it will take for the population of the parasite to become numerically equal to that of the host. The answer is, of course, that this will take place when we have the equation for the population of the parasite equal to that for the population of the host. Putting these two equations equal to one another, *i.e.*

$$ph^t = nh^t - ph^t\,(t-1),$$

we obtain

$$t = \frac{n}{P} \qquad \text{......(6).}$$

From this we may conclude (1) that when host and parasite produce in every generation the same number of offspring, the time required for the control of the host by the parasite will depend directly upon the relation between their initial populations, and (2) that this will hold good whatever the number of offspring produced, *i.e.* whether the populations of host and parasite, considered separately, were increasing or stable.

Suppose, on the contrary, that the number of offspring produced by the parasite is greater than that produced by the host; we can introduce this condition by postulating that $s = ah$, where we have $a > 1$.

Substituting, by a simple transformation we obtain

$$t = \frac{\log\left\{\dfrac{na - n + pa}{pa}\right\}}{\log a} \qquad \text{......(7).}$$

When the reproductive rate of the parasite is greater than that of the host, the time required for control depends not on the ratio between their initial populations, but on the logarithm of this ratio, from which we may conclude that variations in the initial populations will be much less significant than in the case represented by formula (6). Furthermore, this result will be valid whether the host population is stable or increasing and no matter what the actual number of hosts, parasites and offspring happen to be, provided we have $s = ah$ and $a > 1$.

Let us now consider the *implicit limitations* of the example. It is obvious that these cannot be removed by the substitution of symbols for numbers. They depend upon the *type of interaction* selected as a basis for the argument. In order to eliminate them (in so far as this is possible) we must therefore modify our basic hypothesis, or, in other words, our original statement of the case. A few examples will make this clear.

In the original example we supposed that both host and parasite were parthenogenetic female-producing species. Let us now suppose that both sexes are represented. In order to introduce this condition, let l be a factor, such that if $h =$ the number of female progeny produced by each individual of the host, then lh will represent the total number of progeny of both sexes; and, similarly, if $s =$ the number of female progeny produced by an individual of the parasite, then fs will represent the total

number of its progeny of both sexes. The formula for the time, in generations, required for control, when the reproductive rates of host and parasite are equal, then becomes

$$t = \frac{nl}{pf} \qquad \text{......(8)},$$

and

$$t = \frac{\log \left\{\frac{nla - nl + pfa}{pfa}\right\}}{\log a} \qquad \text{......(9)}.$$

When the reproductive rate of the parasite is greater than that of the host ($s = ah$, $a > 1$).

Consider again the important matter of the distribution of the progeny of the parasite among the population of the host. Without discarding our original postulate that the relative distribution of host and parasite remains unchanged, or, at least, does not notably affect the course of events, let us take the two contrasting cases where:

(*a*) Several individuals of the beneficial insect normally co-operate in the destruction of a single host; and

(*b*) One individual of the beneficial insect normally destroys several individuals of the host.

The first case, of course, is that of numerous gregarious parasites such as *Apanteles*, *Microbracon*, *Pteromalus*, etc., while the second is that of the predators, such as Syrphid larvae, beetles, various neuropteroid insects, and so forth.

We can deal with the first case by supposing that the number of parasite larvae normally destroying an individual of the host be $= m$. We can then show that the time required for control is

$$t = \frac{mn - p\,(m - 1)}{p} \qquad \text{......(10)},$$

if the reproductive rates of host and parasite are equal, and

$$t = \frac{\log \left\{\frac{mn\,(a - 1) + pa}{pa}\right\}}{\log a} \qquad \text{......(11)},$$

if the reproductive rate of the parasite is greater than that of the host ($s = ah$, $a > 1$).

To deal with the second case, let us suppose that the number of individuals of the host killed by each individual of the predator be r. The time, in generations, required for control will then be

$$t = \frac{n}{pr} \qquad \text{......(12)},$$

when the reproductive rates of host and parasite are equal, and

$$t = \frac{\log \left\{\frac{na - r + par}{par}\right\}}{\log a} \qquad \text{......(13)},$$

when the reproductive rate of the parasite is greater than that of the host.

Let us now suppose that the increase of the parasite at the expense of the host will produce a difference in the relative distribution of their populations. Two cases may be considered here. Suppose that, at the beginning of the experiment, the relative distribution is such that each individual of the parasite is able to deposit its whole quota of eggs, without running any risk of placing more than one egg in a single host.

If the proportion of parasites to hosts increases, from generation to generation,

and the density of the host population remains constant in spite of the attack of the parasite, then, unless the parasite has the faculty of choosing only unparasitised hosts, the likelihood that eggs will be deposited in hosts already attacked, *i.e.* the *probability of superparasitism*, will increase from generation to generation. If the number of individuals of the host species be N, the number of eggs deposited by the parasite be X, and the proportion of hosts actually parasitised be Y, then the relation between these values can be shown to be

$$Y = N\left(1 - \epsilon^{-\frac{X}{N}}\right) \qquad \text{......(14)},$$

where ϵ is the incommensurable number 2·71828.......

This condition can be incorporated in our algebraical investigation, and it can be shown that the increase in the density of the parasite population, as compared with that of the host, causes a diminution in the effective reproductive rate of the former.

Suppose, on the contrary, that the host population has ceased to increase at the time the parasites begin to act ($h = 1$). In this case, the reproduction of the parasite at the expense of the host will cause an increasing rarefaction of the host population, so that it will become more and more difficult for the parasite to deposit its quota of eggs. Dr W. C. Cook has suggested to me (*in litt.* December 24th, 1928) that the relation between percentage of parasitism and host density might be expressed by the equation

$$a = \frac{K}{1 + \epsilon^{-rt}},$$

where a = percentage of parasitised hosts, K = maximum possible percentage of parasitism, r = rate of increase in parasitism with host density, and t an arbitrary factor which equals 0 when the parasitism equals $\frac{K}{2}$. If the rate of increase in the percentage of parasitism is proportional to the increase in host density, we may write $r = 1$ and our formula becomes

$$a = \frac{K}{1 + \epsilon^{-t}}.$$

By using this formula in our calculations we can investigate the character of the phenomena, following the point where the population of the beneficial insect becomes equal to that of the host.

The preceding method (first described by the author in *C.R. Acad. Sciences, Paris*, 1922) is based on the consideration of the interaction of the parasite and its host as a discontinuous phenomenon. As shown later by Lotka (*Physical Biology*, 1925), it can very profitably be treated as a problem in continuous variation and thus attacked by the method of the calculus. This method, in the opinion of the present writer, is less suitable than the one given above for the exact investigation of experimental results but, though it demands much greater mathematical knowledge, it is more suitable for the edification of a general theory and the formulation of general laws, because of the efficiency of the analytical methods which can be utilised (cf. Péres, *Rev. Gén. des Sciences*, T. XXXVII, No. 10, 1927). From this standpoint the subject has been very thoroughly dealt with by the eminent mathematician V. Volterra, in his paper, published in 1926, on the variation and fluctuation of the number of individuals in species of animals living together (*R. Acc. N. dei Lincei*, Ser. 6, vol. II, Fasc. III). This work was carried out in connection with studies on the interaction of fish populations, but applies to all problems of the interactions of organisms and the regulation of animal numbers.

The foregoing examples, though they simply concern a few isolated points in this vast subject, will serve to show how the significance of the methods outlined can be extended. It is sometimes objected that the construction of a formula sufficiently complex and sufficiently general to correspond to events as they occur in nature would be very difficult. The answer to this is, in the first place, that many factors acting in nature combine to produce simple effects, such as a regular reduction in the efficient rate of reproduction, which can easily be represented, and, in the second place, that no such thing as an *absolutely general* phenomenon of parasite and host interactions really exists. Every case represents a special and, consequently, limited type of this interaction which can often be represented in a relatively simple manner.

Another objection to the use of these methods is that they fail to take account of the irregularity of natural phenomena. The answer to this is that the methods in question have not been developed with a view to the prediction of events over short periods of time, but with a view, on the one hand, to the mapping out of experimental work, and, on the other, to the investigation of general trends. It is true that natural phenomena often appear to be irregular. In many cases, however, this irregularity, though real, is not fundamental. In so far as it depends on *individual variations* it will average out in behaviour just as it does in structure. In so far as it depends on *variation in the environment* it will average out in time, because its fundamental cause, the climate, may be considered as definable over sufficiently long periods. Finally, in so far as the variability is fundamental, as in phenomena which are *essentially* individual in character, it is not matter for science at all, since science deals only with the general.

Though only general trends can be discerned from the formulae, these may be significant, not merely from the scientific but from the economic standpoint. Thus, with the aid of equations (6) and (7), we can investigate the effect of increases in the size of the initial parasite population on the time required for control and show that the expenditure necessary to increase the number of parasites in the initial colony, over and above that required to establish the species, will not produce a proportionate reduction of the time required for control. A slight modification of the method would show that the effect of successive introductions, year after year, of colonies equal to that required to establish the species, is also unimportant, having regard to the increased cost of the work. By a simple transformation we can obtain an equation for the percentage of parasitism after the establishment of a beneficial species and show that the curves of the proportion of parasitised hosts are in all cases very similar, rising for a considerable time very slowly, and rapidly only in the last few generations. From this we may conclude that if we introduce a parasite colony of the usual size in the midst of a large host population, the effect may be imperceptible for a considerable period, even though the experiment is destined to succeed.

It may also be noted that in spite of several modifications introduced the form of the fundamental equations remains essentially similar, which indicates that the trend of the phenomena considered is of the same general character in spite of variations in the various factors which come into play. Such features as are common to all these cases are likely to be of some significance in practical work. They have at least a reasonable probability of being valid, and since, as we have shown, there are no other sources from which we can at present extract any probable rules for the conduct of this work, the rules which can be extracted from the mathematical methods seem at least worthy of attention. It is better to rely on reasonable probabilities than to have no rules at all.

PROCEEDINGS OF THE ASSOCIATION OF ECONOMIC BIOLOGISTS. II

Ordinary Meeting held at 2.30 p.m. on Friday, March 21st, 1930, in the Lecture Theatre of the Royal School of Mines of the Imperial College of Science and Technology, London. The Chair was taken by the President, Dr A. D. Imms, F.R.S.

THE NUTRITION OF FRUIT TREES.

I. Some Effects of Deficiencies of Essential Elements on Fruit Trees. By T. Wallace, M.C., M.Sc., Agricultural and Horticultural Research Station, Long Ashton.

II. Response of Apple Trees on known Rootstocks to Applications of a Complete Fertiliser. By J. Amos, N.D.H., R. G. Hatton, M.A., and T. N. Hoblyn, Hort. Dip., East Malling Research Station, Kent.

III. The Reaction to Potash Fertilisers of Apple Trees in the Field. By N. H. Grubb, M.S.A., East Malling Research Station, Kent.

IV. Some Observations upon the Growth and Seasonal Cycle of Food Reserves in Apple Trees. By T. Swarbrick, M.Sc., Ph.D., Agricultural and Horticultural Research Station, Long Ashton.

I. SOME EFFECTS OF DEFICIENCIES OF ESSENTIAL ELEMENTS ON FRUIT TREES.

By T. WALLACE.
(Long Ashton Research Station, University of Bristol.)

It may be safely stated that at the time when we commenced our nutritional investigations at Long Ashton, knowledge relating to the effects of deficiencies of the essential elements on fruit trees was extremely meagre, and I doubt whether at that time any worker in horticultural research could have described any deficiency symptoms in fruit trees other than certain associated with deficiency of nitrogen and possibly of iron.

This sparsity of knowledge was not due to the lack of previous experimental work on fruit-tree nutrition, but was the result of the failure of the methods employed to provide such information.

Previous to that time, in carrying out nutritional experiments, horticulturists had relied almost entirely on field experiments modelled on the type used so effectively by Lawes and Gilbert in their classical investigations on agricultural crops at Rothamsted.

By far the largest number of such experiments had been carried out in the U.S.A., the only ones of note in this country being those of Bedford and Pickering at Woburn and of Dyer and Shrivell at Hadlow, Kent.

The inadequacy of the experiments in the U.S.A. to throw light on the problems of fruit tree nutrition was stressed by W. H. Alderman, in a paper entitled "The Status of Orchard Fertilisation Experimentation," delivered before the American Society for Horticultural Science in 1919(1), in which he advocated the study of nutritional problems by intensive laboratory methods as a necessary step before proceeding further with experiments in the orchard.

The position reached by the field experiment method was summarised on that occasion by Alderman as follows:

"This seems to be a suitable occasion to pause and consider the work that has been accomplished to date. Within the past two years a number of well conducted experiments, extending over long terms of years, have matured and the results have been made public. So much has been written on the subject that it is a bit difficult to summarise the case, since the evidence does not all point in the same direction. It is somewhat simpler if we first confine ourselves to the points over which there is seemingly no contention.

"1*st*. There are apparently a great many orchards growing upon a variety of soils that will not respond economically to the application of any form of commercial fertiliser, nor of manure. This fact is well established by the work in New York, New Hampshire, Maine, West Virginia and other States.

"2*nd*. Orchards are much more likely to respond favourably if they are given sod mulch treatment than if kept under cultivation. A comparison of the work in the above-mentioned States with that in Massachusetts and Ohio furnishes interesting evidence upon this point, as does also the work in Pennsylvania where the three sod orchards displayed considerably greater gains than the three under cultivation.

"3*rd*. Orchards under starvation conditions usually give a ready response to fertilisation when other treatments (culture, pruning, spraying, etc.) remain unaltered. This is well demonstrated by some of the Ohio, Oregon and West Virginia experiments.

"4*th*. Nitrogen in a readily available form seems to be the only element of plant food that is uniformly a factor in the favourable responses when such are secured.

"These four points, apparently, are about the only outstanding uncontested results of our orchard fertiliser experiments conducted during a period of 30 years or more by 28 different experimental stations. This information is of no small value to the orchard industry and has been well worth working for, but the investigator who is seeking to understand the 'how' and 'why' of the reactions finds only disappointment. There have been those among us who have thought and talked of principles that underlie orchard fertilisation, but so far no principles have been formulated which are capable of application. There are others—probably the majority—who have maintained that orchard fertilisation cannot be reduced to general principles having a wide application. So far, this group has not been disappointed."

In the experiments at Woburn, on the heavy soil at Ridgmont, no significant effects either on the growth or fruiting of apple trees were obtained from dung or fertilisers although, on the same plots, bush fruits, nursery stock and vegetables showed responses to the treatments; on the light sandy soil at Millbrook definite

responses were obtained on apple trees and gooseberries with both dung and fertilisers, provided the latter contained potash.

At Hadlow, on a heavy soil, the outstanding point in the results was the bad effects which followed the omission of potash from fertiliser mixtures when dung was not used.

It will be clear from the above remarks that the field experiments had contributed little to the fundamental problems involved in the nutrition of fruit trees and, in this country, the practical guidance which the results provided was negligible.

In addition to the data obtained through the medium of field experiments, other data relating to the effects of the essential elements in the nutrition of fruit trees had been obtained in various pathological investigations on such problems as "chlorosis" and "die-back," but these were very fragmentary and contributed little to the fundamental knowledge of the rôles of the respective elements in the nutrition of the plants.

Before passing to the specific actions of the various essential elements, it will be useful to refer to some of the reasons why the earlier investigations had yielded such a small amount of knowledge. This we consider to be due largely to the fact that the importance of the inherent properties of the trees and of the various environmental factors which affect tree growth had been insufficiently realised. Workers had been content to carry out experiments in commercial and College orchards with trees about which little was known, such effects as those due to rootstocks being often unsuspected: management factors had usually been adjusted to bring about good growth which in many cases meant that the problems which existed were deliberately overcome by methods similar in effect to the action of certain manurial constituents and thus calculated to mask the effects of these; and the results were usually sought for merely in terms of some empirical growth measurement or the extremely misleading criterion of crop yield, and the conclusions based on such measurements.

Deficiency effects.

The effects which I propose to discuss are those due to deficiencies of nitrogen, potassium, phosphorus, calcium, magnesium and sulphur. The greater portion of the results have been obtained in pot experiments, using sand cultures, in which the various trees and bushes have received their supply of the respective elements solely from chemical solutions while the remainder have been secured in field investigations in which the respective deficiencies have been conclusively proved (3, 4, 5, 6, 7, 8).

In the various experiments, the features on which data have usually been obtained have been as follows: (*a*) times of opening of leaf and blossom buds; (*b*) blossom characters; (*c*) foliage characters throughout the season; (*d*) shoot growth; (*e*) defoliation phenomena—relating to time, method and tints developed; (*f*) conditions of barks; (*g*) yields and characters of fruits; (*h*) root systems.

Nitrogen.

The omission of nitrogen from nutrient solutions produces deleterious effects more quickly than in the case of the omission of any other of the elements considered, and usually, by the end of the first season of treatment, trees receiving this treatment show growth amounts similar to others receiving "water only."

The times of opening of the blossom and leaf buds are delayed, blossom formation is drastically reduced owing to the death of lateral buds, and the flowers are extremely weak. The amount of foliage developed is very scanty and, after a season or two of the

treatment, the trees and bushes usually carry foliage only at the tips of the shoots. The plants thus develop a characteristic "bare wood" appearance. In the case of strawberry plants the number of crowns produced is small. The leaves are relatively small and yellowish green in colour and may develop reddish tints towards the end of the season. It is not unusual for small reddish brown spots to be developed on the leaves. Shoot growth is very drastically reduced, even within the course of one season, and trees, after two or three seasons of the treatment, are usually unable to make appreciable shoot growth. Defoliation is hastened and the tints at defoliation time are reddish yellow. The barks of trees are pale brown in colour. Fruiting is reduced to a negligible amount in a very short period owing to the suppression of the lateral buds. The fruits are small and some idea of the general effect of the treatment on quality in the various fruits may be obtained from consideration of the case of the apple. In varieties which develop red-coloured skins, such as Worcester Pearmain, the colour is greatly intensified and the whole fruit may either assume a vivid scarlet colour or there may be a pale, almost white ground colour with a brilliant red flush. Reducing the nitrogen content of fruits is the only known manurial method of consistently producing high "colour" in our coloured varieties of hardy fruits. In green varieties, such as Lord Grosvenor, the fruits lose their green pigment and in extreme cases are quite chlorotic. The flesh of the fruit is hard and lacks juice, percentage acidity is usually relatively high and the percentages of nitrogen and sugars are low though the ratio $\frac{\text{sugars}}{\text{nitrogen}}$ is high. Flavour only develops slowly after picking, and the fruits are long keepers both in ordinary and low temperature stores.

The root systems are small and in proportion to the dwarfed shoot portions, and it is notable that they consist almost wholly of fine fibrous material.

In practice, various stages of nitrogen deficiency are frequently observed in fruit plantations where cultivation is low and grass and weeds are allowed to grow over the roots of the trees. It is easily remedied by suitable cultivation and by dressings of nitrogenous manures such as nitrate of soda or sulphate of ammonia. The effect of "grass" conditions in producing nitrogen deficiency within the tree is shown in Table I.

Table I.

Showing the nitrogen contents of portions of comparable apple trees under arable and grass conditions. First season of "grass" treatment; variety, Allington Pippin.

Treatment	Leaves of terminal shoots (% N)	Stem portions of terminal shoots (% N)	Fruit (pulp) (% N)
Arable	1·02	0·33	0·046
Grass	0·55	0·24	0·035

Potassium.

In sand cultures, the effects of potassium deficiency are less drastic than those resulting from the omission of nitrogen though, in the field, starvation from the former element is a much more serious problem than from the latter.

In sand culture experiments, deficiency of potassium has frequently advanced the opening of blossom and leaf buds and the blossoms formed have been strong and normal in every way. The number of blossom buds is not drastically decreased as in

the case of nitrogen deficiency and indeed may show some increase due to the fact that, under this treatment, shoots frequently die back and fruit buds rather than shoot buds are developed. This condition is often extremely marked in cases in the field where almost all terminal buds may be fruit buds.

During the early part of each season, the foliage characters are usually quite normal in appearance, but later, usually from the beginning of June, the special symptoms of the shortage of potassium become evident. The green colour at this stage is often bluish green and there may be slight chlorosis near the margins and between the veins. In some varieties of plum, the chlorotic symptoms are strongly marked. In gooseberries, and to a less extent in strawberries, the leaves at this stage may show purple tints, but later, especially in the gooseberry, these tints usually disappear entirely. There is often a tendency for the edges of the leaves to curl backwards towards the under surfaces, but in the case of certain plums, *e.g.* Purple Pershore, the curling effect is in the reverse direction. The leaf margins finally become brown or grey, following the death of the cells in these areas, and the leaves at this stage exhibit the condition known as leaf scorch. This stage is usually well marked by the end of July.

In the case of plants like the raspberry, the browning generally extends between the veins from the marginal areas practically to the midrib. The development of scorch is most marked in hot, dry summers.

In advanced stages of potassium deficiency leaf size is considerably reduced.

In pot experiments, shoot growth is usually somewhat reduced, but in certain cases with apples, during the first two or three seasons, shoot growth in the potassium deficient series has been greater than in the complete nutrient series. The amount of shoot growth in the case of potassium deficiency in this type of experiment appears to be influenced greatly by certain points of technique. Thus if adequate measures are taken to keep the sand medium invariably cool and moist, large shoot growth may result, whereas if the sand is not efficiently protected from temperature changes, shoot growth is greatly curtailed. In cases of potassium deficiency, shoots and even whole branches frequently die-back, and in the field the shoots usually die-back to fruit buds, and in the later stages practically no terminal shoot growth is made, the terminal buds being blossom buds.

Defoliation from potassium deficiency in the field occurs prematurely, following the development of marginal leaf scorch, and this frequently happens in pot experiments. Under the conditions which lead to good shoot growth in cases of potassium deficiency in pot experiments, defoliation may take place *later* than in the case of "complete nutrient" trees. When scorched trees defoliate early in the season, a second crop of foliage is frequently developed. During defoliation the colour of the leaves usually turns direct from green to brown or yellow and highly coloured tints are generally absent. The method of defoliation is remarkable in that, whilst in all other cases examined, it proceeds in the direction from the bases of the shoots towards the tips, the tip rosettes being usually the last foliage retained, in the case of potassium deficiency, defoliation generally, but not invariably, proceeds from the tips of the shoots towards the bases so that the tree retains the older leaves longest. The colour of the barks may be slightly lighter brown than usual in severe cases.

Although potassium deficient trees tend to develop fruit buds rather than shoot buds, the yields of fruit are greatly reduced. The blossoms set fruits quite freely, but

many of the fruits drop during the season, and those which remain are small. The characters of these fruits are worthy of detailed note. In the case of the apple, they may be of dull unattractive appearance, and on keeping in store this appearance does not materially alter, the fruits always appearing less mature than well developed fruits. On the other hand, they may be slightly more highly coloured at the time of picking than high potassium fruits in the case of varieties such as Bramley's Seedling. In store they tend to shrivel and, in most cases examined, they exhibit a most remarkable storage feature in that, in the Ordinary Temperature store, they breakdown considerably *later* than high potassium fruits but in the Low Temperature store they develop breakdown *prematurely*.

The fruits are usually of poor flavour, being subacid and often woody. Chemically they generally show a normal percentage of nitrogen, relatively low acidity, total sugars may be high or low but usually cane sugar is definitely low.

Root systems in the field are usually poorly developed, and badly scorched trees frequently feel quite loose when shaken. In pots, the roots may be quite normal in development, but cases have been observed where the potassium deficient trees fail entirely to form root systems.

Two important points which appear to emerge from our data, both in pot and field experiments, are that potassium deficiency tends to promote physiological drought within fruit trees when the environmental conditions are conducive to drying out, and that the deficiency must usually be considered in relation to nitrogen supply.

Potassium deficiency is the most serious deficiency "disease" of fruit trees in this country and has been of extremely frequent occurrence in the past. It is usually very difficult to remedy by ordinary manurial means in the case of fruit trees when the deficiency effects are strongly developed.

Phosphorus.

The effects of phosphorus starvation of trees in sand cultures after two or three seasons are as severe as or more severe than those resulting from nitrogen deficiency, and the features exhibited in the two cases have many points in common.

The times of opening of blossom and leaf buds are appreciably delayed, and in extreme cases are delayed beyond the times when these occur in comparable trees receiving "water only." Blossom formation is greatly restricted owing to the death of lateral buds and the blossoms formed are very weak. The foliage exhibits very distinctive characters. The amount is very small, and eventually the only leaves developed are the terminal rosettes. Leaf size is much reduced.

It will be noted that all the features described above are similar to those due to nitrogen deficiency.

In the early spring, the leaves are fairly normal in colour, though of rather dull appearance, but they soon become increasingly so and eventually exhibit characteristic purple and bronzed tints over their entire surfaces, the tinting often being accompanied by the presence of brownish spots, which are particularly in evidence in the case of the black currant. After bronzing, areas of the leaves may become dried out.

Trees undergoing phosphorus deficient treatment frequently make excellent growth during the first season of the treatment and show little effect from the deficiency—apparently because of the utilisation of reserves—but, from the second season, shoot growth is generally as severely restricted as from nitrogen starvation, and this condition continues from this point. Defoliation is greatly advanced. It may

occur in early June and *previous* to defoliation taking place in trees receiving "water only" treatment. After defoliation, the trees do not show further signs of growth during the season.

The barks of the trees are slightly paler in colour as the result of the deficiency. The yield of fruit is greatly affected and is similar to that from nitrogen deficiency treatment, as in both cases the reduced yields are due to the suppression of the lateral buds. Unlike nitrogen deficient fruits, phosphorus starved fruits have no desirable commercial qualities but are wholly undesirable objects. The colour is dull with a suggestion of the bronzing which develops so markedly on the leaves. Very few opportunities have occurred for examining the chemical and storage qualities of the fruits owing to lack of crops, but the indications so far are that the fruits are poor keepers and lack character. The root systems are small and in proportion to the small shoot growth, but the character of the root differs markedly from that in the case of nitrogen deficiency, consisting almost wholly of coarse roots and being practically devoid of fine fibrous rootlets. The roots are a characteristic brown colour.

It is of interest to note that, though the effects of phosphorus deficiency in fruit trees in sand cultures are so drastic, no case of proved phosphorus deficiency has been observed in our work in the field, not even in cases where soil phosphorus has been insufficient to grow pasture plants healthily. It thus appears that the fruit plants with which we are concerned are good phosphorus feeders.

Calcium.

No effects on the times of opening of blossom or leaf buds due to deficiency of calcium have been observed and blossoms developed under this treatment have always been quite normal in character. In sand cultures, in the early stages, trees receiving diets deficient in calcium usually carry luxuriant foliage, the leaves being definitely larger than those on trees receiving complete nutrient treatment. This condition is possibly due to "high" potassium and it has been shown that such leaves lose water less rapidly than those from "complete nutrient" series when subjected to drying conditions (2). At a later stage in the treatment, leaf size is more normal and the leaves show breakdown symptoms. The symptoms, in the case of the apple, take the form of patches of dead tissue either near the centres of the leaves or near the margins and, in the latter cases, the brown patches have a reticulated appearance. It will be seen below that similar types of symptoms are developed in the case of magnesium deficiency though, in the latter case, the extent of the breakdown is much more serious. With plants other than the apple, we have not yet been able to produce any distinctive leaf characters. In the large leaf stage, calcium deficiency is also associated with increased shoot growth and, even after three or four seasons of treatment, using various types of fruit plants, we have not been able to effect any significant reduction in shoot growth. Defoliation characters have not shown any distinctive points and the conditions of the barks have appeared normal. Nor have distinctive points been observed to date with regard to the characters of the fruits or the root systems. No case due to deficiency of calcium has been observed in the field.

Magnesium.

The times of opening of blossom and leaf buds have not been affected by omitting magnesium from the diets of various sorts of trees and the character of the blossom has always been normal. Extremely definite effects are produced on the foliage from

a very early stage. In the first stages of magnesium deficiency, leaf size is inclined to be increased and usually remains of at least normal size over two or three seasons. In some instances, however, leaf size has been greatly decreased from the treatment, notably in an experiment in which the apple Stirling Castle was used (5).

Each class of plant appears to develop a specific type of breakdown symptom. In the case of the apple, the usual symptom is the death of a relatively large patch of tissue near the centre of the leaf around the midrib, and this condition we have termed "blotch," but the patches may occur in a haphazard manner on any portion of the leaf and in some varieties, are frequently developed near the margin. The symptom, it will be noted, is similar to that shown by calcium deficiency but has always been much more severe in our experiments. In the later stages of the deficiency, the green colour of the leaf may be slightly pale.

In the case of the black currant, the central portion of the leaf turns purple and this coloration develops over practically the whole of the leaf surface until finally usually only a narrow marginal band remains green. The leaf at this later stage becomes curled towards the under surface.

Raspberry leaves first develop a yellow patch of colour in the centre, a fairly broad band around the margin remaining green. A narrow yellow band later develops around the margin, leaving a narrow green band between the two yellow areas.

Strawberry leaves develop coloured areas in the same manner as those of the raspberry but the centre patches show reddish or purplish tints.

The characters which develop on gooseberry leaves differ from all the above-mentioned cases in that they invariably commence around the margins. Broad red marginal bands are first developed and these gradually broaden and spread towards the centre. As this proceeds, the red tints fade to yellow, the final colour being a faded, yellowish red.

In all cases of magnesium deficiency, defoliation is premature and follows closely on the development of the breakdown symptoms. It may occur extremely early in the season in severe cases and, when this occurs, fresh foliage is developed which usually becomes affected with the deficiency characters at an early stage. No effects on the colour of the bark have been noted from the treatment.

Although the trees blossom normally, the yield of fruit is seriously affected where defoliation is severe and in such cases the fruits never reach a mature condition but remain woody, deficient in juice and sugars and are typically immature fruits. Apples from this treatment have retained this immature character after several months in store.

The root systems have generally been well developed and normal in character but a considerable amount of killing of the root fibres has frequently been observed.

No serious cases of magnesium deficiency have so far been encountered in the field but the leaf characters herein described are frequently observed. In such cases they have been often interpreted as denoting an ample supply of potassium, since it has been shown by the writer that magnesium deficiency can be induced by high potassium feeding (4). In certain cases, the respective tints appear to be characteristic of certain varieties—*e.g.* the black currant variety, Baldwin, and the gooseberry variety, Whinham's Industry, and can only be suppressed by drastic deficiencies of other elements.

Sulphur.

The observations made relating to sulphur deficiency are much less extensive than in the cases of the elements previously described and, at the present stage, it can only be said that trees receiving sulphur deficient diets have usually made slightly smaller growth than those receiving a complete nutrient and, in general, have exhibited characters similar to those of trees receiving a slightly restricted supply of nitrogen. Thus the colour of the foliage has been slightly pale green, defoliation has been hastened to a slight extent, and during defoliation, brilliant orange tints have been developed. Root systems have appeared normal in character. No case of sulphur deficiency has been recognised in the field.

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II. THE RESPONSE OF APPLE TREES ON KNOWN ROOT-STOCKS TO APPLICATIONS OF A COMPLETE FERTILISER.

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(With 4 Text-figures.)

The project.

When the main rootstock trial of Bramley's Seedling and Worcester Pearmain apples was planted in January 1920, it was decided to include a sufficient surplus of trees on certain rootstocks for the purpose of making an elementary test of manuring as compared with no manuring. Since many of the manurial trials of previous workers with apples in the field had yielded indecisive results (1), it was planned, in the first instance, to confine the issue to the simple one of comparing the effects of an annual dressing of organic manures—more or less completely "balanced"—with the results obtained by withholding the same, and merely ploughing into the ground, from time to time, some non-leguminous cover crop for moisture-retaining purposes.

The lay-out.

The ground—a loam of variable depth over the Hythe beds—was, at the time of planting, considered to be exceptionally even. Mechanical and chemical analyses of these plots were made in 1913, and the details are given in an appendix. It had been

cropped from the autumn of 1913 to that of 1916 with Royal Sovereign strawberries, which were treated uniformly. A year previous to their planting, the whole ground was occupied by grey peas which had received a dressing of 2 cwt. superphosphate per acre.

The following table (I) shows the manurial and cultural treatments of the ground from 1908–9 until the time the trees were planted. It will be noted that the dressings were predominantly nitrogenous and phosphatic. Potash manures were almost unobtainable during the period from 1914 onwards, though a light dressing was given in 1918–19. From the autumn of 1919 onwards the differential treatments began on the plots.

Table I.

Treatment of ground for previous 11 *years.*

Year	Manurial treatment (rates per acre)	Cropping
1908–9	Dung 30 tons; gas limed	Potatoes and mangel
1909–10	—	Wheat
1910–11	—	Seeds ley
1911–12	Nitrate of soda and nitrate salts	Oats
1912–13	Superphosphate (35 %) 2 cwt.	Grey peas
1913–14	Dung 10 tons; light crop mustard; meat and bone 4 cwt.	Strawberries
1914–15	Meat and bone 6 cwt.; nitrate of soda 1½ cwt.	Strawberries
1915–16	Dung 16 tons	Strawberries
1916–17	Litter and strawberries ploughed in	Wheat
1917–18	Dung, London, 25 tons + wheat stubble. Superphosphate 4 cwt.; sulphate of ammonia ¾ cwt. up drills	Potatoes
1918–19	Dung 12 tons; flue dust 10 cwt. Superphosphate 4 cwt.; sulphate of ammonia 1 cwt.; sulphate of potash, 1 cwt. up drills	Potatoes

Approximately 2½ acres of this land was devoted to the project. At the time it was recognised that great difficulties in cultivation, etc., would have to be faced, since ground was not available for "guard" rows. On any chessboard arrangement the manures would inevitably be carried over by implements from one plot to another. Again, the experience of Pickering, in manuring alternate trees and starving the others, suggested that the question of root range must be carefully considered. Hence, in view of the simplicity of the issue, and the limited facilities then at command, it was decided to have only two plots (1¼ acres each), adjacent to one another, the one manured and the other starved. It is hardly necessary to point out that such a lay-out is open to severe criticism; on the other hand, both the issue and results have been so clear cut that they seem to justify the presentation of the data. In addition, some 200 trees, upon which records were taken individually, are involved in the trial; each one can strictly be regarded as a plot in itself. Thus the number of reduplications is considerable, indeed never less than 8 and, in the case of the starved plot, usually 16.

The material.

The two varieties, Bramley's Seedling and Worcester Pearmain, the buds of which were taken from trees of good history, were thought likely to respond very differently to manurial treatments. Bramley's Seedling represented a most vigorous growing type of green cooking apple, robust in constitution, whilst Worcester Pearmain, grown to perfection, is a highly coloured mid-season dessert fruit with considerable external "quality." Its constitution is generally considered weak, being especially susceptible to canker and scab.

Both these varieties were budded upon eight distinct varieties of known root stocks, raised in the Station's nursery. With a single exception—that of No. X stock—all the others belonged either to Group (*b*) "Semi-dwarfing," *i.e.* Nos. II, III, V, VII, or to Group (*c*) "Vigorous," *i.e.* Nos. I, IV, VI.

No. X was at the time thought to belong to the "very vigorous" Group (*d*) since, in the early years, when worked with Lane's Prince Albert, it made exceptional growth. Later, this variety was shown to approximate to an "incompatible" stock with some varieties, especially with Bramley's Seedling, whilst with other combinations its early heavy cropping propensities have checked its growth in later years (2).

It should be stated that observations upon many of the experimental plots (3) have shown that several representatives (Nos. V, II and VII) of the so-called "semi-dwarf" Group (*b*) possess in common not only a somewhat similar type of root system, but the potentiality for producing leaf-scorched trees—given appropriate external conditions. This physiological disturbance of the tree, proved by Mr T. Wallace of Long Ashton to be an indication of potash deficiency (4), is far less frequent and acute where rootstocks Nos. I, and VI are in use.

Thus, at the outset, the two plots included trees with very different latent potentialities, the significance of which has only become apparent as the records thereon have accumulated.

All the trees were planted as one year olds. They were weighed at time of planting, unfortunately not individually but in bundles of five trees—the mean weights being deduced from these figures. Table II gives these averages for sets of eight trees upon each stock on the manured plot and for similar sets of sixteen trees on the unmanured plot.

As will be seen, without exception, the trees planted on the unmanured plot

Table II.

Average weights at planting in ounces per tree.

On rootstock No.	Worcester Pearmain		Bramley's Seedling	
	Manured	Unmanured	Manured	Unmanured
I.	9·5	9·8	11·5	13·1
II.	6·3	6·4	5·3	8·3
III.	4·4	6·4	5·6	6·5
IV.	5·8	6·3	6·3	8·5
V.	6·7	7·3	9·0	10·4
VI.	7·3	10·8	10·3	11·8
VII.	6·9	8·6	7·8	9·4
X.	8·0	8·5	6·0	9·9

happened to be sturdier than those on the manured. This is explained by the fact that the average weight for all the "plantable" trees on each stock was first taken, and those round about the mean were first selected for planting. When the unmanured plot was reached, trees somewhat above average size had to be used.

In other words, quite unintentionally and as a result of faulty planning, the unmanured plot started with decidedly better trees, the most notable cases being those of Worcester Pearmain on No. VI and Bramley's Seedling on No. X rootstocks.

Planting.

The Bramley's Seedling and Worcester Pearmain were planted alternately at 15 feet square—the former being intended for "permanents," the latter as "filler" trees.

Each set of either variety upon a single rootstock was divided into two, and planted in two locations on the plot. There was no definite attempt made to interplant trees upon strong and dwarfing rootstocks or to plant them in any selected order according to growth potentialities.

The weather was more favourable for the planting of the manured than it was for some of the trees on the unmanured plot—a heavy rain having supervened—but there is no evidence in the next few years that the manured plot subsequently benefited in any way thereby.

Treatment of the plots.

The treatment of the plots immediately prior to planting and subsequently, to date, is detailed in Table III.

Owing to the limited space available and to the financial exigencies of the Station, it was found necessary to intercrop both plots for three years with a common vegetable crop. In January 1922 these were replaced by a single row of raspberry canes which

Table III.

Manurial treatments and intercropping of the two plots since planting.

Year	Manurial treatments (rates per acre)	
	Manured plot	Unmanured plot
1919–20	Shoddy* 30 cwt.; soot 66 bushels; lime 10 cwt.	Nil
1920–21	Dung 15 tons	Mustard
1921–22	Shoddy 30 cwt.; lime 10 cwt.; fish meal 5 cwt.	Mustard. Lime 10 cwt.
1922–23	Dung 15 tons; bone siftings 2½ cwt.	Cabbage
1923–24	Shoddy 30 cwt.; meat and bone 5 cwt.; rape as green manure	Rape
1924–25	Dung 15 tons; muriate of potash 4 cwt.; rape dust 6 cwt.	Nil
1925–26	Shoddy 30 cwt.; meat and bone 4 cwt.; Peruvian guano 2 cwt. (on raspberries)	Nil
1926–27	Sulphate of potash 3 cwt.	Nil
1927–28	Sulphate of potash 3 cwt.; meat and bone 6 cwt.	Nil
1928–29	Sulphate of potash 3 cwt.	Nil

* A black wool shoddy analysing 7 to 8 % ammonia was used throughout.

formed the main variety collection at the Station until they were all grubbed in December 1928. The same varieties were planted upon both plots. A single black currant bush was planted in 1923 between the trees in the tree rows, the variety Baldwin on the manured plot and the French varieties on the unmanured plot. These were also grubbed in 1928.

Whilst there is no denying that this intercropping was, to say the least, unfortunate, some of the crop records obtained therefrom gave a useful indication that the condition of the soil on the two plots after the differential treatments in the early years, differed at least in so far as small fruits and annual crops were concerned.

Records taken.

Before studying the tabulated data obtained from these trees, it will be well to explain the nature and methods of the records taken thereon.

During the early years, all the trees were "leader tipped" according to a uniform scale, and all lateral shoots above a certain maximum length were "spur pruned." This treatment has been continued to date on all trees of Worcester Pearmain on root-stocks Nos. I and II on both plots. As long as this treatment has been continued, it was found possible, in most cases, to measure all the new shoot growth in each individual tree. When, for cultural reasons, it became urgent to stop leader tipping, as in the case of Bramley's Seedling on all stocks, other expressions of tree vigour had unfortunately to be solely resorted to, such as the average height and spread of the head of the tree, and the annual girth measurement of the main stem taken at a fixed point. These latter observations were begun sometime before the wood measurements were discontinued.

All fruits have been counted, graded and weighed. The grading has in some cases been for colour and scab blemish as well as for size.

In several years records of the amount of leaf scorch upon certain sets of trees have been taken on both plots. Some records of canker and apple mildew are also available for comparison.

Manurial treatments subsequent to planting.

Table III also shows the manurial treatments on the two plots from the time of planting up to the present day. It will be noticed at once that, as was the common horticultural practice at that time, in the early years the dressings on the manured plot were in the main nitrogenous. Up to the end of 1926, the only potassic dressing given to the trees was a dressing of muriate of potash in March 1925. On the unmanured plot, four green crops were ploughed in up to the end of 1925, but two of these were only very light dressings owing to the failure of the crops to grow satisfactorily.

About this time it became clear, first through the work of Wallace (4) and subsequently through the field trial of Grubb (5), that potassic dressings were likely to be essential to the proper growth of fruit trees in the field, and that the phenomenon known as leaf scorch, which was becoming increasingly prevalent in certain varieties, was closely connected with improperly balanced manurial applications, and in particular a deficiency of this element.

Since 1926, therefore, an effort was made to make good this deficiency, and in each year a dressing of 3 cwt. of sulphate of potash per acre has been applied to the fully manured plot, while the nitrogenous dressings have been somewhat reduced. During this period the starved plot has received nothing at all.

The effect on tree vigour.

(*a*) *The first six seasons.*

As has been pointed out, at the time of planting the trees on the starved plot were, if anything, somewhat sturdier than those on the fully manured plot; and during the next five seasons' growth the only differences in vigour of which it is possible to be certain, are in favour of the unmanured plot. Table VI shows the position at the end of this period. It will be seen that, with the exception of the trees of Bramley's Seedling on Nos. VII and X, there was no significant difference between the two plots, either in total wood growth or in girth of stem at the end of this time. In several cases the differences were still slightly in favour of the starved plot; and in the two cases mentioned, *i.e.* Bramley's Seedling on Nos. VII and X, the trees on this plot were still definitely ahead.

Table IV.

Comparison of the vigour of trees on the manured and unmanured plots at the end of six seasons' growth.

	Bramley's Seedling				Worcester Pearmain			
	Total wood growth (in m.)		Girth of stem (in mm.)		Total wood growth (in m.)		Girth of stem (in mm.)	
Rootstock No.	Manured	Unmanured	Manured	Unmanured	Manured	Unmanured	Manured	Unmanured
(*b*) V.	53·5	53·6	213	212	46·4	48·3	153	154
VII.	54·3	61·7	**193**	**209**	50·9	57·6	142	148
II.	60·4	56·4	214	210	58·3	52·2	162	155
III.	61·6	57·0	213	206	57·3	55·9	162	155
(*c*) VI.	57·8	61·7	204	209	43·1	56·1	144	154
I.	66·1	70·0	217	221	55·5	57·2	153	159
IV.	72·4	67·6	218	215	—	—	153	157
(*d*) X.	**37·3**	**46·9**	**154**	**185**	51·0	54·2	154	154

For the sake of simplicity in this and most of the tables to follow, the averages per tree alone are given. The individual tree records have, however, all been analysed and are, as usual, available for inspection by those interested. In every case where the difference between the two plots is significant (*i.e.* the odds against the difference being due to chance are greater than twenty to one), the two means thus differing are in black type in the table.

(*b*) *From the seventh to the eleventh season.*

(i) *Different response of the two varieties.*

From the seventh season onwards, differences which can be attributed to manurial deficiency began to make their appearance, whilst it is interesting to note that these differences became apparent in the season immediately following the first dressing of potash, both experience of the comparatively slow working of such a dressing upon apple trees and the symptoms of the plot as a whole suggest that this may not be the only deficiency. Tables V and VI give the history of the vigour of the two varieties as expressed by girth increments, on each of the eight rootstocks in the trial, from the seventh season (1925) up to the end of 1929. Comparing the two tables, it is evident

Table V.

Annual girth increments in millimetres per tree from the seventh to the eleventh season.

BRAMLEY'S SEEDLING.

Rootstock No.	Plot	No. of trees	Actual girth at the end of 1924	(S.E.)	Girth increments 1925	1926	1927	1928	1929	Actual girth at the end of 1929	(S.E.)
(*b*) V.	Manured	8	213	(*4·8*)	43	53	60	49	57	475	(*9·1*)
	Unmanured	16	212	(*3·6*)	36	51	49	41	45	433	(*6·6*)
VII.	M.	8	193	(*7·6*)	44	50	55	40	34	416	(*13·7*)
	U.	14	209	(*3·2*)	33	48	43	31	23	386	(*7·6*)
II.	M.	8	214	(*5·9*)	42	54	61	47	54	471	(*8·7*)
	U.	16	210	(*5·1*)	36	56	55	41	43	441	(*10·5*)
III.	M.	8	213	(*5·9*)	48	58	65	49	54	487	(*6·1*)
	U.	15	206	(*5·9*)	40	51	50	36	29	413	(*12·2*)
(*c*) VI.	M.	8	204	(*5·7*)	48	58	64	45	49	468	(*12·8*)
	U.	16	209	(*2·2*)	47	57	55	45	45	459	(*6·2*)
I.	M.	8	217	(*6·0*)	43	56	57	38	45	455	(*13·2*)
	U.	16	221	(*5·1*)	39	56	54	45	42	456	(*9·2*)
IV.	M.	8	218	(*4·6*)	47	59	59	50	45	478	(*6·0*)
	U.	16	215	(*2·6*)	43	55	50	40	28	429	(*4·8*)
(*d*) X.	M.	8	154	(*5·7*)	35	39	44	36	39	347	(*11·2*)
	U.	16	185	(*4·1*)	33	44	45	36	32	374	(*5·1*)

Table VI.

Annual girth increments in millimetres per tree from the seventh to the eleventh season.

WORCESTER PEARMAIN.

Rootstock No.	Plot	Actual girth at end of 1924	Girth increments 1925	1926	1927	1928	1929	Actual girth at end of 1929
(*b*) V.	Manured	153	33	33	40	35	43	337
	Unmanured	154	30	31	35	29	35	314
VII.	M.	142	27	32	25	23	27	276
	U.	148	29	31	26	22	21	277
II.	M.	162	33	41	40	34	45	355
	U.	155	26	39	32	27	28	307
III.	M.	162	34	39	42	36	43	356
	U.	155	30	34	33	29	30	311
(*c*) VI.	M.	144	29	35	31	26	28	294
	U.	154	31	40	40	33	39	338
I.	M.	153	29	38	33	28	31	312
	U.	159	28	36	30	30	33	316
IV.	M.	153	33	35	34	30	34	319
	U.	157	32	35	33	27	30	314
(*d*) X.	M.	154	25	33	30	29	30	301
	U.	154	26	32	26	25	25	287

that there are more differences in favour of the manured plot in the case of Bramley's Seedling than in that of Worcester Pearmain. Referring to the last column, which gives the actual girth of the main stem at the present day (March 1930), it will be seen that in four cases (*i.e.* on rootstock Nos. V, VII, III and IV) the Bramley's Seedling trees on the manured plot are now well ahead of those on the unmanured, and in two more cases the difference appears to be in favour of manuring (Nos. II and VI). Where the rootstock is No. X, it will be noted that on both plots the trees are much smaller than those on the other seven rootstocks. This stock appears to be almost incompatible with Bramley's Seedling, and while trees on the manured plot are certainly smaller than those on the unmanured, it seems possible that this is not connected with the manurial treatments since, as shown in Tables II and IV, the latter were well ahead at the outset.

In only two cases (Nos. II and III) have the trees of Worcester Pearmain on the unmanured plot fallen very far behind those on the manured plot and in three cases (Nos. VII, I and IV) the trees show practically no difference. In one case (No. VI) the trees on the unmanured plot are still definitely ahead. Table II again shows this was strikingly the case at time of planting, and Table IV still suggests a difference in their favour. It should be noted, however, that on the manured plot the trees on this stock are now practically the smallest on the plot, although by no means the smallest at planting. Undoubtedly there are two or three exceptionally bad trees in this set. This may possibly be accounted for by the shallow planting of some of these particular trees, which is obvious on inspection.

The cause of this more definite response of Bramley's Seedling to starved conditions is probably varietal and may in part be closely connected with the greater susceptibility of this variety to leaf scorch. It seems likely therefore that a deficiency of potash would affect the vigour of this variety more markedly than the less susceptible Worcester Pearmain.

(ii) *Different response upon different rootstocks.*

The tables are arranged, for the sake of convenience, with the stocks grouped together under the original grouping of Lane's Prince Albert for vigour (6). It will be seen at once that, in spite of variations in relative vigour between the eight rootstocks from the original grouping, the stocks which are first affected by the starved conditions are to be found in the so-called "semi-dwarfing" Group (*b*).

With Bramley's Seedling all the trees on these four rootstocks (Nos. V, II, III and VII), which have been subject to starved conditions, have fallen well behind those on the fully manured plot, while only one (No. IV), has done so in Group (*c*). No. IV rootstock is notable for inducing heavy crops at an early stage.

With Worcester Pearmain, trees on three out of the four rootstocks on Group (*b*) (Nos. V, II and III), have fallen behind, while none of those in Group (*c*) are as yet affected.

Fig. 1 demonstrates one such comparison; here the annual increments in cross-section of the stem are compared for Bramley's Seedling on the two plots, on the semi-dwarfing stock No. V and the vigorous No. I.

On No. V the trees on the manured plot are annually making greater increments than those on the starved plot, whereas on No. I there is little to choose between the trees on the two plots in vigour; indeed, in the only year where a marked difference

occurred (1928), that difference was in favour of the unmanured plot. This was probably close y connected with the cropping of the trees, since, as will be shown, although the vigour of trees on the vigorous rootstocks, as shown by the measurements of stem girth,

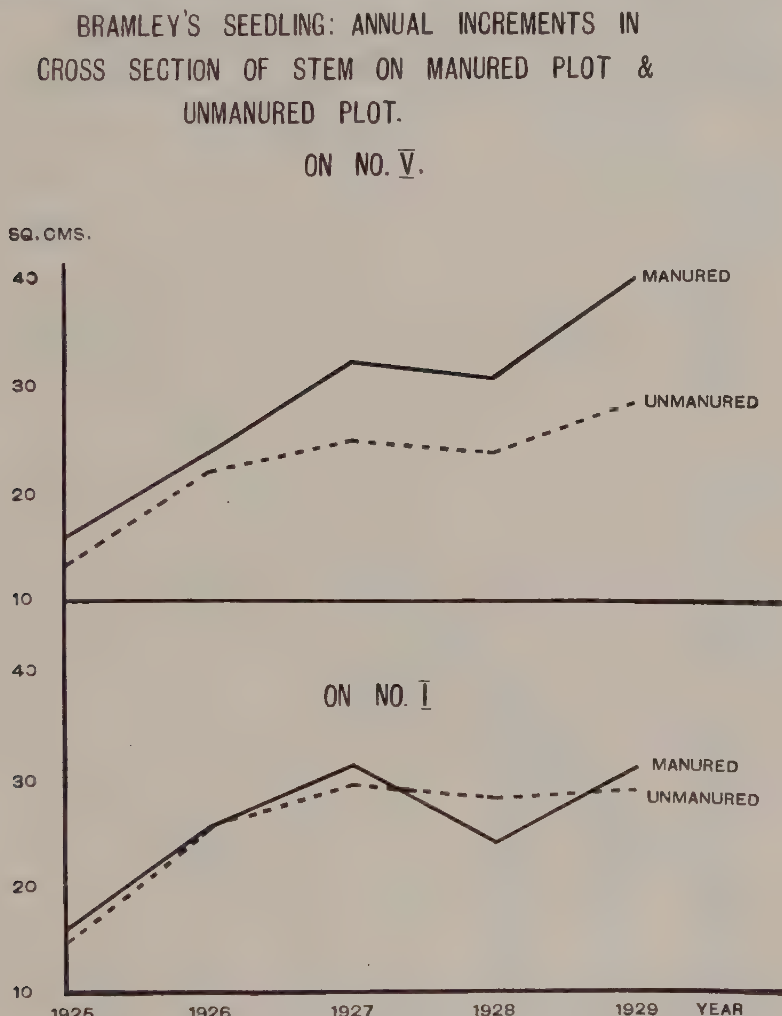

Fig. 1.

has not yet been greatly affected, there have been very definite differences in cropping between the two plots.

An interesting result of the earlier influence of starvation on trees on the semi-dwarfing stocks is demonstrated in Fig. 2, where the average wood growth of Worcester Pearmain on Nos. I and II is compared for the two plots up to the present day. It

will be seen that No. II (Doucin) induces a bigger tree of this variety than No. I (Broadleaf), under balanced manurial conditions; where manures are withheld on the other hand, No. II resumes its position as a semi-dwarfing rootstock, as compared with No. I, which under these conditions produces the bigger trees.

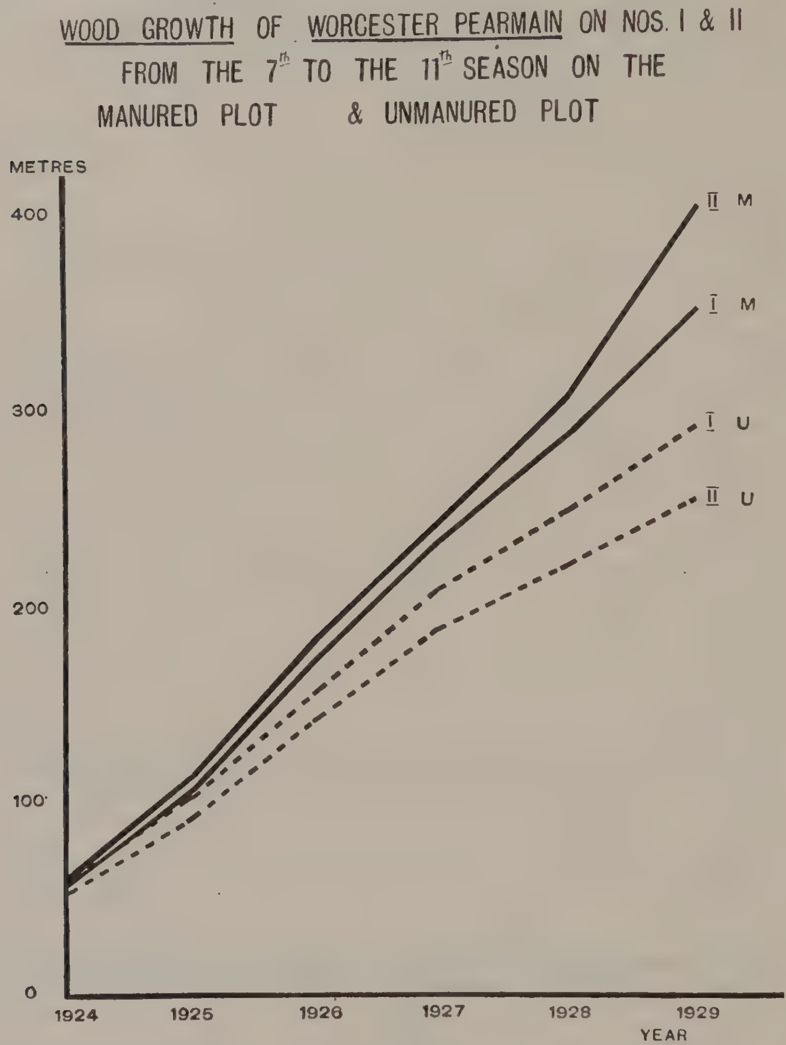

Fig. 2.

It will be noted by reference to Table V that the same result has occurred with Bramley's Seedling.

Before leaving the question of vigour, one or two clear cases of differential effect should be noted.

While Worcester Pearmain on No. IV has behaved like the other members of the vigorous Group (*c*), this rootstock under Bramley's Seedling has induced trees which have responded to starved conditions in exactly the same way as those on members of the semi-dwarfing Group (*b*).

It should be remembered that although this rootstock, itself a dwarf, produces as a rule vigorous trees, it also displays many of the characteristics of a dwarfing or semi-dwarfing rootstock, such as early heavy cropping, and the typical swelling at the union of scion and stock so characteristic of this group. It is also known to be a rootstock especially suited to Worcester Pearmain.

Worcester Pearmain on No. X, while by no means forming an incompatible combination, seems to behave in much the same way as Group (*b*). This variety on No. VI has so far shown no evidence of manurial starvation in its vigour, indeed, as has been pointed out, the trees on the unmanured plot, which were heavier at the start, are still significantly larger.

As has been observed, measurements of height and spread have also been taken on these trees, and, while allowing for the effect of pruning upon these characteristics, they bear out in general the results as shown by girth of stem and wood growth.

The effect on cropping.

(*a*) *Worcester Pearmain.*

Of the two varieties in the trial, the first to come into cropping was Worcester Pearmain. This variety produced small crops in the sixth and seventh seasons (1924 and 1925). In these two seasons little difference was to be noted between the two plots in actual weight of crop harvested, as shown in Table VII, although those on No. IV, the heaviest cropping trees, certainly produced more fruit on the manured plot. In 1926 the crop failed and the first crop of any size was harvested in 1927. In this year, on the manured plot the four heaviest cropping sets of trees (*i.e.* those on Nos. II, III, I and IV) produced a significantly heavier crop than on the unmanured

Table VII.

Cropping of Worcester Pearmain on manured and unmanured plots in pounds per tree.

Rootstock No.	Plot	1924	1925	1927	1928	1929
V.	Manured	0·9	3·6	16·9	25·8	41·0
	Unmanured	0·4	3·6	14·5	22·3	35·5
VII.	M.	2·7	**3·4**	19·8	22·6	**47·3**
	U.	2·8	**5·1**	18·3	20·8	**26·8**
II.	M.	**4·2**	6·1	**25·7**	**41·3**	45·4
	U.	**2·5**	8·0	**17·2**	**23·7**	37·2
III.	M.	1·9	5·5	**30·3**	**41·5**	41·9
	U.	1·9	5·3	**20·3**	**24·7**	42·3
VI.	M.	1·9	2·6	18·2	26·7	52·3
	U.	1·7	3·4	17·1	24·5	54·5
I.	M.	3·6	7·3	**34·6**	**34·9**	**68·3**
	U.	2·4	7·2	**20·6**	**25·6**	**53·6**
IV.	M.	**6·1**	**8·4**	**38·8**	**42·0**	**79·1**
	U.	**3·8**	**5·1**	**22·6**	**25·6**	**57·1**
X.	M.	**2·7**	3·7	19·7	26·9	**54·6**
	U.	**1·2**	3·5	19·7	25·1	**40·9**

There was practically no crop in 1926.

plot, and this was repeated in the following year. In 1929 the trees in the vigorous group all produced larger crops than those in the semi-dwarfing group, and, of these, Nos. I and IV, still had the larger crops on the manured plot. Although the trees on No. III had more or less equal weights of fruit on the two plots in this year, the general tendency in the semi-dwarfing group was in favour of manuring, and this is also the case with the trees on No. X. Generally speaking, with the exception of Nos. V and VI over the whole period, the trees on the manured plot have produced the larger crop.

The bigger trees of this variety on No. VI on the unmanured plot have already been noted, and this is probably the reason why the weight of crop is not smaller on this plot. No. V is a rootstock which seems to produce small crops with all varieties, and in this case even balanced manuring does not seem up to the present to have benefited the trees to any extent.

(*b*) *Bramley's Seedling.*

This variety produced very little fruit on either plot prior to 1928. Up to the end of 1927, as is shown in Table VIII, there was no difference which could be attributed to manuring.

In 1928 these trees produced their first heavy crop, and in this year in every case, except No. X, the trees on the manured plot had definitely the larger crop.

Table VIII.

Cropping of Bramley's Seedling on manured and unmanured plots.

Rootstock No.	Plot	Total No. of apples to 1927	1928 (lb.)	1929 (lb.)
V.	Manured	55	71	79
	Unmanured	64	32	62
VII.	M.	85	114	199
	U.	92	75	101
II.	M.	72	119	85
	U.	73	81	107
III.	M.	39	88	115
	U.	46	58	106
VI.	M.	46	74	168
	U.	48	41	146
I.	M.	74	119	137
	U.	53	64	118
IV.	M.	72	95	234
	U.	72	69	149
X.	M.	5	20	71
	U.	26	44	92

In 1929 the trees on the unmanured plot developed a large number of short blossoming spurs as compared with the manured plot. In consequence, the actual numbers of fruit set on the starved plot was much greater, and although, as will be shown, the actual fruits on this plot were very much smaller, in one case (No. II) the trees on the unmanured plot actually had a somewhat larger weight of fruit, and in three other cases (Nos. III, VI and I), the weight harvested was not significantly in favour of the manured plot.

Table IX.

Size of fruit, as shown by number of apples to the pound, on the manured and unmanured plots.

Rootstock No.	Plot	Worcester Pearmain 1925	1927	1928	1929	Bramley's Seedling 1928	1929
V.	Manured	5·0	5·5	6·0	5·0	—	3·2
	Unmanured	5·3	6·4	5·8	6·2	—	4·6
II.	M.	4·5	4·9	5·3	4·5	3·4	2·7
	U.	4·8	5·6	6·7	6·3	4·2	3·9
I.	M.	4·4	4·6	5·0	4·8	3·3	2·7
	U.	5·0	5·5	6·1	6·0	4·1	4·3
X.	M.	4·2	5·0	5·6	5·2	3·1	3·2
	U.	4·8	5·2	5·7	6·1	3·6	4·1

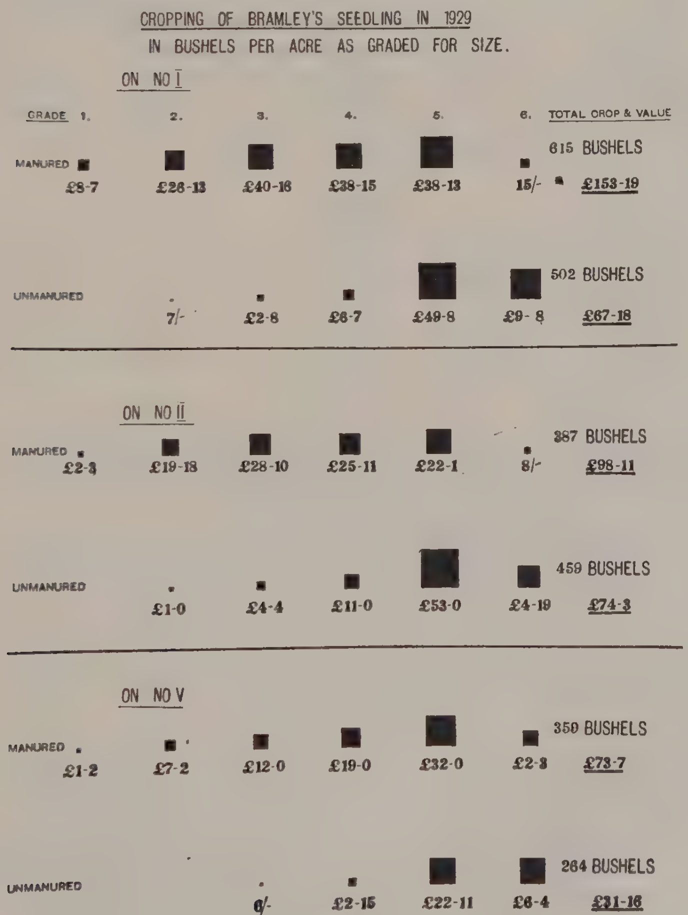

Fig. 3.

Effect on size of fruit.

Although, in all cases, significant differences are not apparent in total weight of crop, very definite differences in size and quality of fruit have become evident. As far back as 1925 (Table IX), the fruits of Worcester Pearmain appeared somewhat larger on the manured plot, as is indicated by the average number of apples to the pound. Since that time, in practically every case, the manured plot has produced bigger fruits, not only of Worcester Pearmain, but also of Bramley's Seedling. This was even apparent where the fruits were thinned.

This fact naturally makes a very big difference to the value of the crop. In every year the fruits have been graded for market. Fig. 3 shows the relative crop in bushels per acre, in each of the six size grades, for Bramley's Seedling on Nos. I, II and V on the two plots in 1929. An average value per bushel for each grade has been calculated from the prices received throughout the season, and from this it is possible to compare the total value of the crop under each different treatment after marketing expenses have been deducted. It is obvious that, although, in one case (No. II), the greater weight of fruit was harvested on the unmanured plot, the fact that practically the whole of this crop was to be found in the two smallest grades makes the smaller crop on the manured plot of greater value. Thus on the starved plot there were 459 bushels having a value of £74. 3*s*. and on the manured plot there were only 387 bushels, but the value was £98. 11*s*.

This diagram once more emphasises the poor return produced by trees on No. V when compared with other rootstocks, since here not only is the actual crop smaller but the fruits themselves are very much smaller, even when properly manured. Thus, on the manured plot, the value of the crop on No. I was £154 per acre, while that on No. V was only worth £73 per acre, and on the unmanured plot the value was £68 on No. I, as against only £32 on No. V. A table could be presented showing much the same type of performance for Worcester Pearmain.

Another interesting comparison may be made between the behaviour of Worcester Pearmain on these two rootstocks. Whilst the growth on those on No. V is adversely affected by starvation, the fruit crop is not materially improved by manuring. Exactly the reverse is the case of the trees on No. I.

The fact has been frequently commented on that No. V stock not only scorches badly itself in the stool beds, but also produces trees which are always the worst affected by this trouble. Indeed where potash has been applied, the trees on this stock are known to recover less rapidly than on other rootstocks. This may have contributed to the above result, although it is by no means certain that it is the whole cause; since, whatever the treatment, this stock always seems to give trees of a comparatively poor fruiting performance.

Effect on fruit quality.

Not only have the trees on the unmanured plot produced small fruits in the last two or three years, but they have also lacked that less readily measurable characteristic, quality. By means of the normal grading of the fruit for market into three grades, "Extra Fancy," "Fancy" and "C" Grade, it is possible to show that, apart from size, this difference is a real one. Fig. 4 shows the percentage of the total crop in each of these three grades for Bramley's Seedling in 1929 on Nos. I and II on the two plots. It is at once clear that a greater proportion of high quality fruit has been

obtained from the manured plot. It is a little difficult to describe the condition of the fruits which is responsible for this, since actual colour is not a quality which is of importance in Bramley's Seedling. However, it is quite certain that there has been a general lack of condition and a tendency for the fruit to bruise more easily in handling which was probably partially responsible for this result. The trees of Worcester Pearmain do not show this lack of quality on the unmanured plot quite so markedly as

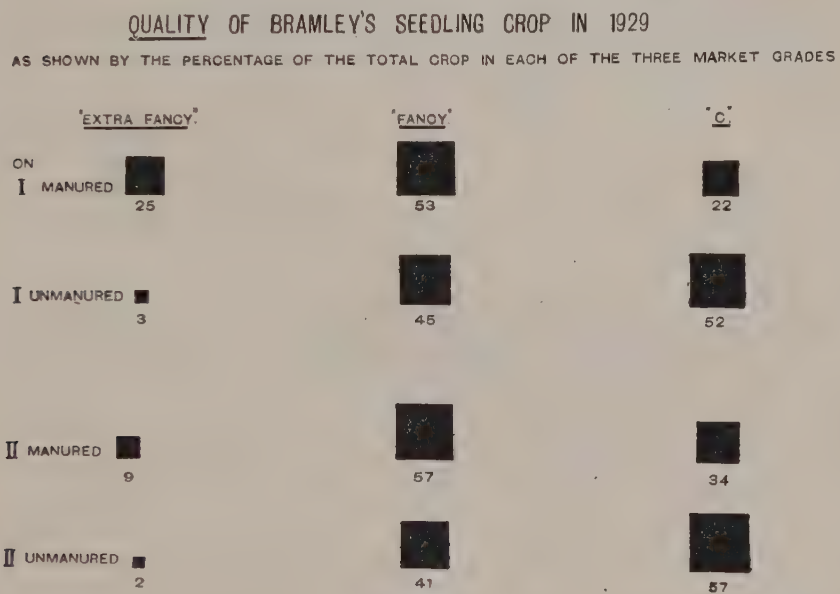

Fig. 4.

those of Bramley's Seedling. The fact that colour is here the most important qualitative feature is partially responsible. Although the coloured apples on the unmanured plot were undoubtedly of a duller and less attractive red than those on the manured, it was not possible to show this difference in the grading. Again, the fruits in this case were marketed immediately, and thus the bruising factor was avoided. The question of the storage quality of fruits from these plots is not dealt with here.

Effect on disease.

Another qualitative factor which has contributed to this striking result has been the marked difference in the amount of scab found on the apples on the two plots. For both varieties on all stocks there was a much greater amount of scab on the starved plot. Counts of the scabbed fruits were made in 1929 by Mr M. H. Moore, and his estimates for Worcester Pearmain are shown in Table X. Moore (7) has described elsewhere his technique for the estimation of this infection, and there is no doubt about the significance of these results either for the first or second picking in the single year (1929) when this record was obtained.

As has been pointed out before (3), the amount of leaf scorch on the unmanured plot has been considerably greater than on the manured plot, and at the same time

Table X.

Occurrence of scab on the fruits of Worcester Pearmain in 1929 on manured and unmanured plots.

Rootstock No.	First picking		Second picking	
	Manured	Unmanured	Manured	Unmanured
V.	3·8	5·6	5·7	7·4
VII.	6·0	11·8	8·8	14·3
II.	4·0	9·3	7·8	8·6
III.	4·4	12·5	7·7	15·6
VI.	4·7	11·1	8·1	13·8
I.	6·0	6·5	6·4	8·4
IV.	7·0	16·2	8·7	18·3
X.	5·1	7·4	4·9	9·4
General mean	5·13	10·03	7·26	11·98
S.E. of general mean	0·32		0·44	

Table XI.

Leaf scorch on Bramley's Seedling and Worcester Pearmain on rootstock Nos. I and V on unmanured plot. July 15th, 1929.

Rootstock No.	Bramley's Seedling (%)	Worcester Pearmain (%)
I.	9·2	7·2
V.	24·7	15·6
S.E. of difference	3·6	4·5

N.B. There was no leaf scorch on the manured plot at this date.

certain varieties of rootstock have given trees which have been more susceptible to this trouble throughout.

An estimation of the percentage of the leaves affected was again made in July 1929. At this time there was no leaf scorch on the manured plot, but on the unmanured the trouble was already advanced. Table XI shows the amount on the trees on Nos. I and V for both varieties at this date. It will be seen that in both cases there was considerably more on the trees on No. V, some 25 per cent. of the leaves of Bramley's Seedling being scorched on this stock as against 9 per cent. on No. I. The amount of scorch on Worcester Pearmain was not so great (and varied greatly from tree to tree), but the difference between the two stocks is still apparent.

In previous years, when the drought factor had not so seriously intervened to cloud the issue, the comparative amount of leaf scorch on the two stocks was much more obvious.

General indications of starvation.

In addition to the measurable differences such as vigour, cropping, size of fruit, etc. already described, there were certain other indications that the starved trees on the unmanured plot were suffering from some manurial deficiency, which have been obvious to the eye at any rate during the last two seasons. Thus the appearance of the trees during the whole season was one of debility; in addition to much leaf scorch, the leaves were

thin in texture and of a pale sickly green, the new wood growth was stunted and the fruit obviously small and of poor quality. Even in the winter it is possible to distinguish the worst affected trees by the many short bifurcated growths at the terminals.

On the other hand, the manured plot showed a striking contrast. Here there was practically no leaf scorch, even in the last season, when this trouble was accentuated by the prolonged drought which undoubtedly affected the growth of all varieties.

These trees appeared throughout strong and healthy. The foliage was of a deep healthy green, thick in texture, and the fruit was of excellent finish and quality.

Thus, the answer to the simple question of whether apple trees in the field respond to fertilisers has been given for East Malling conditions at least, within the first ten years of their life, and apple trees do respond sooner or later to such dressings, though the extent of that response will depend upon the variety, and the rootstock upon which it is worked.

The second stage in the experiment will be an attempt to trace and remedy the deficiencies in the field.

Summary.

1. A simple experiment, wherein a plot of apple trees receiving applications of a balanced manure has been compared with a similar plot which has received nothing but applications of non-leguminous green crops, has now been conducted for eleven seasons.

2. On land which was in "good heart" at the time of planting the trees showed no response to manuring during their first six years' growth.

3. From the seventh year onwards to the present date, very definite differences began to appear between the two plots in vigour, cropping, size and quality of fruit and health of the trees.

4. Of the varieties used, Bramley's Seedling has suffered from the starved conditions to a greater degree than Worcester Pearmain.

5. The same variety budded upon different rootstocks shows a marked differential response; trees on rootstocks in the "semi-dwarfing" group have suffered earlier and more markedly than those on the vigorous group of rootstocks.

6. It is also demonstrated that a rootstock inducing really bad performance, such as the poor crops and small fruits produced by trees on Doucin Ameliore (No. V), still gives bad trees, even when subjected to balanced manuring.

Appendix.

(1) *Typical chemical analysis of soil at East Malling.*

	Soil	Subsoil
Moisture	1·067	1·066
Organic matter	3·386	2·033
Nitrogen	0·1047	0·1400
Carbonate ($CaCO_3$)	0·354	0·352
Potash (K_2O)	0·2951	0·316
Potash available	0·024	—
Phosphoric acid (P_2O_5)	0·1117	0·073
Phosphoric acid available	0·0296	—

(2) *Typical mechanical analysis of soil at East Malling.*

	Soil	Subsoil
Hygroscopic moisture and soluble	2·955	2·460
Fine gravel	3·193	3·357
Coarse sand	39·733	30·503
Fine sand	21·084	24·696
Silt	15·944	17·03
Fine silt	6·872	8·145
Clay	6·194	9·367
Organic matter	3·386	2·033

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THE REACTION TO POTASH FERTILISERS OF APPLE TREES IN THE FIELD.

By N. H. Grubb.

(*East Malling Research Station.*)

(With 3 Text-figures.)

Apple leaf scorch was already causing some concern at East Malling as long ago as 1919, the oldest trees on the Research Station being then in their sixth year from planting. It was considered serious enough to justify a close study of its severity, and the mapping of two affected areas tree by tree. Although this early study did not then lead to any result, it provided useful data for comparison with later observations, after the potash treatment was adopted.

The adoption of the potash treatment followed, of course, Wallace's discovery through pot-culture experiments that leaf scorch is usually a symptom of potash deficiency (1). The results obtained in our trial were reported in considerable detail in 1928 (2).

The plot used for the trial is the "Pruning Plot," on which a comparison of the effects of different methods of pruning has been in progress for some thirteen years. The arrangement of the potash trial, from the experimental point of view, is somewhat crude, the plot having been merely divided into two equal parts, a "treated" and an "untreated" half. But although the arrangement is so elementary, we can present the results with some confidence; for in the first place, the half of the plot chosen for treatment showed consistently more leaf scorch before the treatment began, and the trees were making noticeably less progress; and in the second place, with twenty sets of trees, comprising fifteen varieties, each treated as regards pruning in four different ways, we are able to make a large number of comparisons between groups of treated and untreated trees.

In addition, we fortunately possess individual tree records of growth and cropping for four years *before* the potash treatment was begun; we can therefore compare the behaviour of the trees on the two halves of the plot both before and after the treatment began, and can show in some detail how the treatment has affected their behaviour.

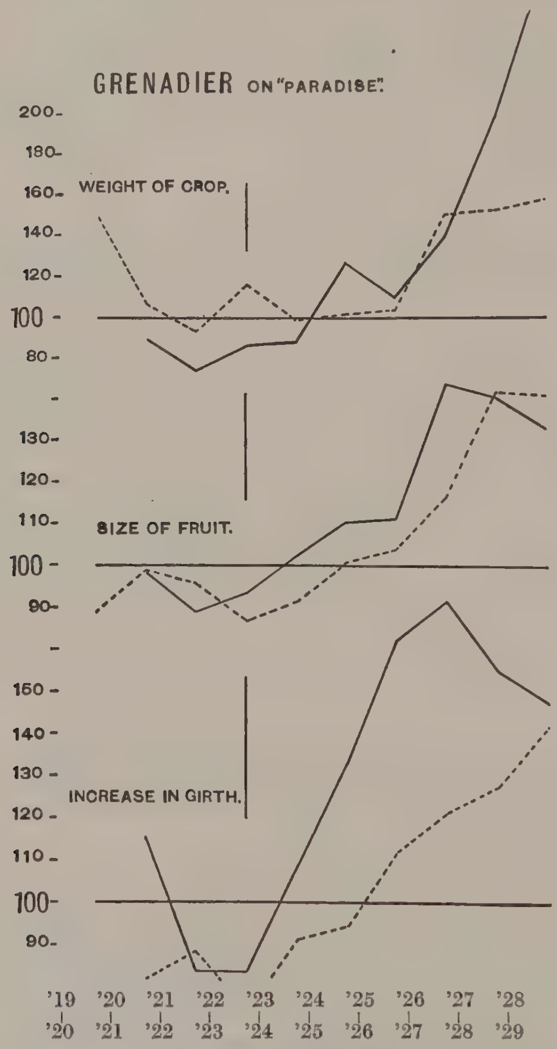
The first potash dressing was given early in 1923. The following winter it was omitted, but it was resumed in 1925, and has been continued annually since then. With two exceptions, the dressing has consisted of 4 cwt. per acre of sulphate of potash—a fairly heavy dressing. The first application, in 1923, was 2½ cwt. per acre. In 1928 the dressing was cut down to 2 cwt. per acre, but was restored to 4 cwt. in 1929. In 1928 the greater part of the "untreated" plot received a dressing of 4 cwt. per acre, to show how far one application (not repeated) would go in bringing the untreated trees up to the improved performance of the treated trees.

All other manures and fertilisers, and in fact treatments of all kinds, have been given identically to both halves of the plot. Prior to 1923 fairly generous dressings of nitrogenous artificials, usually following dung or shoddy, had been given annually; this may well have intensified the leaf scorch. But in 1919 the whole plot had received a dressing of 15 cwt. per acre of "flue dust," which would no doubt to some extent counteract the effect of heavy nitrogen feeding.

The results to date, in cropping, size of fruit and increase in girth of stem, of three varieties are shown in the graphs. The three varieties cover a wide range; Grenadier has always (where not treated with potash) had more severe leaf scorch than any other variety in the plot and thus gives an extreme result; Beauty of Bath is the most vigorous variety of the fifteen, and has been the slowest to crop—the trees having never yet borne anything like a full crop; and Worcester Pearmain is recognised as being more resistant to leaf scorch than most varieties. Beauty of Bath and Grenadier occur on that part of the "untreated" plot which was still left untreated in 1928—they have been potash-starved since 1919; whilst Worcester Pearmain received the one dressing of potash on the "untreated" plot in 1928. These three varieties fairly represent the whole collection, and illustrate most of the points of interest.

Previous to the beginning of the treatment, as the graphs indicate, the trees on the (subsequently) treated plot compared poorly with the remainder, in cropping, size of fruit and girth increase; they were either behind or falling off, relatively, in all respects. In every case there is a marked improvement, beginning about the time of the first application of potash.

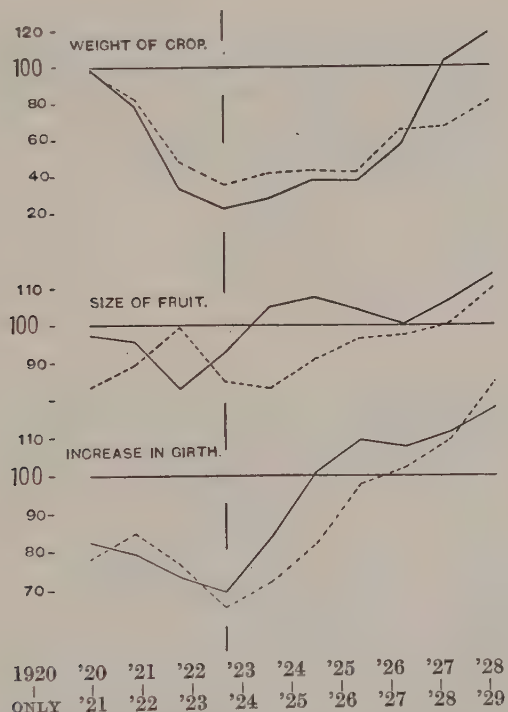
At the same time, the leaf scorch was noticeably reduced. Surveys of its severity were made for several years in succession. No clear reduction could be seen, either by eye judgment, or by sample leaf counts, after the first potash application; after the second, in the summer of 1925, several varieties showed a slight reduction of leaf



scorch, and generally improved foliage; whilst after the third application the improvement was distinct over almost the whole plot. The graphs indicate that in many cases the turning point was reached actually with the first application; but nothing short of a numerical record would have shown it; eye judgment is not nearly accurate enough.

Although we have obtained this very definite response to potash, in cropping, size of fruit, growth and quality of foliage, we have by no means eliminated the leaf scorch. In the very dry summer of 1929 many trees on the treated plot showed severe leaf scorch, though scarcely ever as severe as the worst cases on the untreated plot. This is reflected in the graphs for Worcester Pearmain in the relatively falling rate of girth increase, and reduced fruit size; several other varieties give similar curves. In a few cases, both in 1928 and 1929, the trees on the treated plot appeared to have as much or more leaf scorch as those on the untreated plot. There are several possible explanations of this fact.

BEAUTY OF BATH.

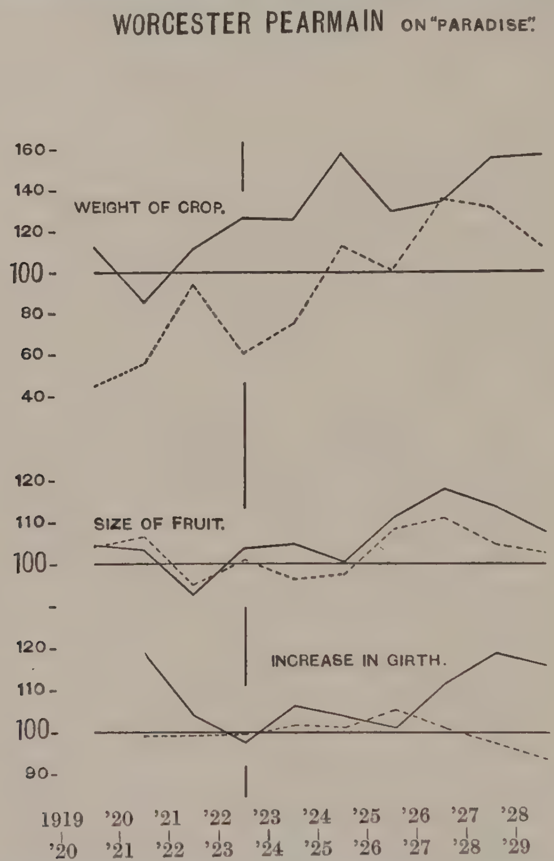


In the first place it is quite possible that the potash has stimulated somewhat excessive cropping, and since a heavy crop always makes leaf scorch more severe (other things being equal), the treated trees may at times suffer from leaf scorch as severely as the untreated. In a few cases it seems to be a fact, also, that the heavy cropping stimulated by the potash has checked growth; growth and leaf scorch are so closely related, negatively, with cropping, that one can hardly expect all to be equally stimulated by the potash.

In the second place the one application of 4 cwts. per acre given to part of the "untreated" plot in 1928 should just have begun to show its effect. The fall in the

curves for Worcester Pearmain, which received this application, compared with those for Grenadier and Beauty of Bath, which did not, points in this direction.

But a third possible cause—the severe drought—seems likely to be in itself a sufficient explanation. If potash applied to the surface, and cultivated in, is very slow in penetrating to the deep sub-soil, there is likely, after six or seven annual applications, to be a considerable accumulation of potash in the top soil of our treated plot. And if one of its first effects, as seems likely, is to stimulate rapid root growth, it is



certainly possible that our treated trees, especially after the very wet summer of 1927, had become largely dependent on a mass of comparatively shallow roots. A severe drought which prevented these roots from functioning might well have a worse effect on the treated than on the untreated trees.

Other data, besides those used in the graphs, confirm the general deductions. The increase in growth of the treated trees is shown equally by the weight of wood removed from the pruned trees in pruning, by the number of new shoots, and by the height and spread of the trees.

A question on which more data are needed is the effect of potash (if any) on blossom bud formation and on blossom "setting." Since the number of fruits has in most cases been increased by the potash treatment, either the number of blossom buds, or the proportion of blossoms which produce fruit, or both, must be increased.

There is no longer any doubt that in certain cases the "set" of fruit has been greatly increased by the potash. The most conspicuous case has been that of Rival in 1928. In 1927 the treated trees of Rival bore more than twice as much weight of fruit as the untreated trees; as a result, in 1928 the blossom of the treated trees was somewhat scanty compared with that of the untreated trees; yet the number of fruits was approximately equal on the two sets of trees, and the weight of fruit was greater on the treated trees, owing to its greater size. It seems, then, that the effect of potash on fruit bearing is at least partly due to a better setting of the blossom.

On the other hand, it seems probable that in certain cases, particularly amongst the more severely pruned trees, where the crop was somewhat *reduced* after the first two or three applications of potash, the effect may have been actually due to a reduction of blossom bud formation caused by the potash. If a young tree, not fully in bearing, is invigorated in some way, its blossom bud production may presumably be for a time reduced. Since potash has had an invigorating effect on our trees, it may well have tended to delay the cropping of those which had not already borne heavy crops. True, this effect does not show in the case of Beauty of Bath—tipped or untipped—which as I said has been our slowest variety to crop; but it does seem to show in the case of the leader-tipped trees of Norfolk Beauty, Annie Elizabeth, and Grenadier on "Crab" (not those shown in the graph), all of which have been slow to crop.

The possible effects of potash on fruit colour and maturity are more difficult to study. So many factors affect fruit colour, directly or indirectly, that it is very hard to disentangle them. Fruit colour records have been taken of one or more varieties each year, and the results are in many cases contradictory. There appears, however, to be a simple explanation of these variable results. It has frequently happened that the fruit of the leader-tipped trees has been better coloured from the *untreated* plot, whilst that from the untipped trees has more usually been better coloured from the *treated* plot. This is intelligible if we suppose that the stimulation of growth on the treated plot has made the foliage of the tipped trees relatively denser, in comparison with the untreated plot, than that of the untipped trees. Eye judgment would suggest that this is the case. On this view, the potash-fed trees should produce the better coloured fruit, except where the denser foliage, resulting from more vigorous growth, shades the fruit and prevents full colouring. In at least one case (Newton Wonder in 1928) it was obvious, just before picking, that the fruit exposed to the light was much more highly coloured on the treated plot, whether on tipped or untipped trees. But the effect was masked when the fruit was picked, owing to the large number of almost green fruits from the lower branches of the treated trees. The effect would probably have been more pronounced had the trees (both treated and untreated) been regularly summer pruned, so as to give the fruit a better chance to colour fully.

Observations on various diseases have indicated a somewhat indefinite result. Fruit growers have sometimes claimed that they have prevented scab (on the fruit,

presumably) by potash treatment. Our trees do not yet give any such indication; such records as we have made actually show slightly more scab on the fruit from the *treated* trees. This may, however, be only an indirect effect of the potash, due to the much denser foliage of the treated trees.

This observation is not necessarily in conflict with the fact, noted in the last paper, that a comparison of the completely starved plot with the regularly manured plot at East Malling shows markedly more scab on the starved plot. The "untreated" plot in the potash trial has been fairly generously manured, except with potash, and is not at all on a par with the completely starved plot.

Apple canker, again, does not yet seem to have been reduced by the potash treatment. The only variety in the plot which has recently cankered badly—James Grieve—has consistently shown more canker on the treated plot, both before and since the treatment began. Since the most vigorous trees of James Grieve usually show the most canker, it seems quite possible that the potash treatment may have slightly increased their susceptibility.

Apple mildew is the only fungoid disease which does seem to show a reduction on the treated plot. Records are as yet somewhat scanty, but it is clear that Newton Wonder, Lane's and some other varieties, are now distinctly freer from mildew on the treated plot, which appeared to be slightly more affected by the disease before the treatment began.

Finally, an observation made in 1928 suggested a curious and unexpected influence of the potash. In that year the whole plot, treated and untreated, was sprayed, after blossoming, with lime sulphur at 1 in 100, plus 4 lb. of lead arsenate paste per 100 gallons. Two varieties, Rival and Lane's Prince Albert, showed some injury and considerable leaf fall. In the case of Rival, the leaf fall was very much heavier on the untreated plot; a rough estimate suggested that there were three or four times as many leaves on the ground under the untreated trees as under the treated trees. In the case of Lane's the difference was slight, and would probably have escaped observation, but for the striking difference in the case of Rival.

Summary.

The application of sulphate of potash over a period of years to part of the "Apple Pruning Plot" at East Malling has resulted in:

1. A great reduction of "leaf scorch."
2. Greatly increased growth of the trees.
3. A great increase in weight of crop and size of fruit.
4. In some cases better "setting" of the blossom.
5. In a few cases a probable reduction of blossom bud formation.
6. Heightened fruit colour, except where increased foliage shades the fruit.
7. No reduction of disease, except probably apple mildew (and "leaf scorch").
8. In one or two cases, foliage more resistant to "spray injury."

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EXPLANATION OF GRAPHS.

The graphs show the relationship, in biennial periods, between the trees of three varieties on the treated and untreated parts of the plot.

Figures for untreated trees taken as 100 (heavy horizontal line).

Continuous line ———————— = untipped trees.
Broken line – – – – – – – – – – = tipped trees.

Heavy vertical line shows first biennial period including an application of potash.

SOME OBSERVATIONS UPON THE GROWTH AND SEASONAL CYCLE OF FOOD RESERVES IN APPLE TREES.

By Thomas Swarbrick, M.Sc., Ph.D.
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Introduction.

All physiological investigations in horticulture are fundamentally questions of nutrition. Differences in rates and directions of metabolic activity are the causes of our observed differences in external growth phenomena. The nutrition of fruit trees occupies a large place in physiological investigations in horticulture because nutrition in its major aspects is so closely related to and affected by environment.

The major problem in horticulture from a physiological standpoint is a growth problem of a very special order. It is not the problem of the timber merchant and the forester who are primarily interested in the amount of wood, but it is the problem of obtaining in our trees that kind, amount, quality and distribution of growth which will give us fruit as a main end product of metabolism.

In view of the fact that tree growth as we see it with our eyes is an index or a resultant of certain physiological or nutritional states which precede it, we are confronted at once with the great task of elucidating and separating the various factors which together underlie and determine growth responses as we know them in practice. This is the task of correlating internal antecedent conditions with external growth features so that from external characters we may correctly diagnose internal conditions. Thus shall we be able to initiate corrective treatments for physiological disorders which will be grounded in scientific knowledge. The quest of the physiologist at the moment is the attempt to correlate growth characters with their internal antecedents, so that we may correctly diagnose tree conditions and nutritional needs.

The necessity for stressing this view point is shown by a consideration of the fact that the effect of any cultural or manurial treatment upon *yield* is always an indirect effect. The effect of the treatment is primarily an effect upon the metabolism of the plant. This has its effect upon tree growth which in turn affects yield and quality of fruit. Hence if we judge our results by yield only we are very likely indeed to miss entirely the significant diagnostic features revealed by manurial and cultural treatments. In any study of tree nutrition the primary consideration appears to be the effect of the treatment upon *growth* responses, and through this upon yield. The

attempt must be made to understand growth features in terms of their internal metabolic antecedents and the environmental conditions which produce them.

This view point and method of recording results, if more universally adopted, would go far towards clearing the issues in many of our manurial and pruning experiments. The effects of a cultural or manurial treatment upon yield will often be determined by purely local conditions, and by the condition of the trees when the experiment was started. In the majority of very detailed pruning and manuring experiments that are reported from time to time, we are not able to evaluate the results or formulate a general principle because we are not given the external growth features of the trees before, during and after the treatments. In many cases a careful description of the effects of the treatments upon the tree growth would at once suggest a rational nutritional basis for observed results in yield. We often miss the significant features of experimental treatments, because yield is used as a main criterion rather than a combination of yield along with a detailed *description* of the changing growth responses of the trees under treatment. An experiment in fruit tree nutrition has value in a fundamental way only when its influence upon the manufacture, accumulation and use of food materials is known, and is correlated with observable external growth responses.

Pending such a close study and unified attack, we may proceed a long way towards our goal by using the small amount of knowledge that we have. An exact knowledge of internal conditions is not always necessary to a practical diagnosis. There are certain growth features which answer very well for purposes of practice if it is remembered that they are not the factors we are looking for, but are an index to certain desirable internal conditions of metabolism.

With a view to extending our knowledge of the internal metabolic features associated with external growth characters of fruit trees, investigations have been initiated at Long Ashton relating to the movement of food reserves in apple trees, and in the present paper an account is given of the seasonal cycle of starch in apple trees in relation to the observed seasonal growth cycle.

The seasonal cycle of starch storage in relation to growth phenomena.

In previous work upon the healing of wounds in woody stems (1, 2) the writer was led strongly to the opinion that it was essential to learn much more of the seasonal cycle of food reserves in the plant, and of the relation of these fluctuations to such well-known growth phenomena as the start and cessation of extension shoot growth, the initiation of flower buds on spurs and on one-year wood, to the "set" of flowers, and the initiation of radial growth in the stems and roots.

The seasonal cycle of starch within the woody parts of the tree above ground level was first investigated. This cycle is briefly as follows.

It is convenient to commence the description with the conditions in January. Starch at this time is more or less abundant in all the living cells of the above ground parts *except the cambium* cells and the apical meristems. The medullary ray cells of the xylem, the xylem parenchyma, the phloem parenchyma and cortex are particularly well filled with starch.

During February there is a very pronounced disappearance of starch from the phloem region of the one- to five-year-old branches. This disappearance is most marked and is followed by a *complete reappearance of the starch in late February and early*

March. If the February examinations had been missed the starch disappearance in that month would not have been suspected from the March examinations.

References in the literature showed that many of the quantitative estimations of carbohydrates made by the chemical investigators into the carbohydrate reserves in apple tissue also showed a marked drop in the "starch" fraction during the January-February period. Many of the workers were inclined to relate it to the "cold" of that period. There are, however, strong reasons for suggesting that it is a phenomenon more deeply rooted in plant economy. It is here suggested that it is a change which is associated with the ending of the "rest period." Beyond this we cannot go at the moment but the case is briefly discussed elsewhere (3, 4).

The next major event is the swelling of the terminal and flower buds of the trees in spring. Beyond the starch disappearance in February there are no internal changes apparent in the starch reserves prior to this bud swelling. This does not mean that there are none but that I could *observe* none. Many things seem to *follow* closely upon bud swelling and breaking.

At the time when the buds break into leaf or flower the cambium of the younger branches is swollen and translucent in appearance. This swollen, turgid, gelatinous, translucent appearance of the cambium which is observed subsequent to bud break is in marked contrast to the dry whitish appearance during winter. At the "swollen" stage of the cambium the bark will "slip" but no new xylem or phloem is being added except immediately underneath the terminal expanding buds. As soon as shoot growth is really started, starch begins to disappear from the phloem and outer xylem region of the younger shoots. This disappearance begins first under the terminal buds, and works basepetally, *i.e.* the starch disappearance is from above downwards. Following this starch disappearance *down* the stems is the initiation of new xylem. The initiation of new xylem is also basepetal.

Thus, during spring, we have an influence of developing buds upon the disappearance of starch, and the initiation of new xylem formation, an influence which is manifested from above downwards. Due to this basepetal development the initiation of new xylem upon the branchless part of the tree trunk is often several weeks later than the same phenomenon upon the one-year-old shoots. This downward trend of starch disappearance and new xylem formation is continued down the bole and out into the roots.

Starch disappears completely during early spring from the one- to three-year-old branches but may not be complete from the inner ring of a four-year-old branch or the two inner rings of a five-year-old branch and so on. The starch disappearance is almost complete from the cortical tissues over the whole tree. Due to the continued starch depletion above described, there is a minimum starch content in the whole of the tree from about the middle to the end of June. At this time shoot extension growth is very rapid and vigorous, and apparently the newly formed leaves are not yet able to supply more food than is being used in the building up of the new tissues. When the growth rate of the extension shoots begins to fall off, starch begins to accumulate again in the tissues from which it disappeared in spring, and it does so *from above downwards.* The new starch deposition begins in the new shoots and works downwards. Similarly, radial growth also ceases first in the one-year-shoots and the cessation of radial growth proceeds in a basepetal direction. Thus, of the above-ground parts of the tree, the collar region is the last part to lose its starch in spring and is the last part to com-

mence radial growth. It is also the last part to be re-stocked with starch in autumn and is the last part to cease radial growth. Quite often radial growth may continue at the ground level until winter sets in.

Here one may diverge to raise some of the economic and practical questions that touch upon the above facts of plant growth. There is first of all the question of winter hardiness. Fortunately this is not one of our main problems here in England, but it is a vital question in Canada. Winter injury is found at three main points in a tree:

(1) The crotches;
(2) The collar region;
(3) The roots.

It has been shown above that the collar region of a tree is a region of late growth in autumn. Similarly the crotches are regions of late growth, and it will be shown later that the roots are the regions of latest growth of all. Obviously winter injury is found in these regions because they are still succulent at the time of the injury. The problem of winter injury is largely solved by growing varieties that have short growing seasons and so cease growth early.

Secondly, there is the question of the differences in behaviour between trees of similar make-up growing under similar conditions, except they have in one case a short leg and in the other a long leg—the differences in fact between the behaviour of bush trees and standards. These differences are, no doubt, due in large measure to the time taken for the downward trends to reach the roots.

Finally, there is the relation of the observed starch cycle to the initiation of flower buds in apple trees. It has been recently shown(5) that, at Long Ashton, flower buds may be found developing upon spurs of apple trees during early July. In this connection it was stated earlier in this paper that there is a period of minimum starch content during the May-June period. Since the factors which determine flower bud formation must be operative long before the anatomical evidences of floral parts are visible, we are led to regard the May-June period as critical in flower bud initiation. The amount of starch and carbohydrate used in growth and flowering in spring becomes very important, because the amount of carbohydrate left as an unused residue in the tissues at this time may be an important factor in determining flower bud formation. The well-known residual effects that are exhibited in fruit trees may be associated with the completeness or otherwise of the starch removal in spring.

There yet remains for discussion the question of the starch cycle and growth periodicity of the roots. Unfortunately the study of roots is much more difficult than that of stems. During 1927–8 some observations were made upon roots similar to those made upon stems and reported above. Root material is by experience far more variable than stem material and the difficulty of interpretation is thereby increased. However, it is clear that the wave of cambial activity which moves down the stem passes out into the roots, and the period of radial growth in the main roots does not begin until about August, and from then it may continue until January. The intensity of the wave diminishes as it passes outwards, and many cases were observed where it never reached the root extremities.

The starch cycle in roots is difficult to interpret. There are large differences in the amount of starch in different roots of the same tree at the same date. It would appear that, in the main, the wave of starch disappearance from the stem is continued out

into the roots. Over and above this downward *disappearance* there is the appearance of a much less intense wave of starch disappearance, which begins at the root tips and works upwards. At some point these two waves appear to overlap. This may be the cause of the very variable starch content of the roots as a whole. In any case roots are rarely found without starch altogether, and the interpretations are made with great difficulty. The reappearance of starch in roots is most certainly connected with the wave of starch deposition that comes down the stem from above.

The periodicity of new root elongation growth also tends to complicate the interpretation of the starch content of roots. Work at present in hand seems to show that there are two main periods of root extension growth. In established trees one is in spring and starts *before* shoot growth begins. This period continues until early summer. The other period, which is by far the more important one, begins about August and continues until well into winter.

I should like at this point to stress the importance from a nutritional point of view of these two periods in root growth. One occurs *before* and during blossoming and the other occurs in late summer and autumn, and is active after leaf fall. Both these are periods when we are normally not doing much in the way of cultural practice. The whole question of our present cultural and manurial practice becomes somewhat debatable, in view of the periodicity in root growth outlined above. It would seem to be one of the next big problems in the study of fruit tree nutrition.

It is exceedingly difficult in so little space to give an adequate outline of the cycle of tree growth into which we must fit our interpretation of nutritional studies in fruit trees. Many details are omitted which are interesting and probably important.

The part played by developing terminal buds in initiating the cycle of changes in starch and cambial growth has been mentioned. By the artificial removal of all the buds of a shoot the above changes may be prevented. When this is done, the shoot may remain alive for a year or more, but it will remain "dormant" as far as its starch and cambial conditions are concerned, even if the branch remains attached to an otherwise normally functioning tree.

This question of terminal bud dominance penetrates even farther, for the *kind* of bud that terminates a shoot determines the rate and the relative dominance of the subsequent activities in the subtending shoot. There is a difference in the subsequent growth activities in a shoot depending upon whether it is terminated by a vegetative or by a flower bud. Vegetative buds, in *comparison with flower buds*, are followed by a less rapid and complete starch disappearance and a much earlier and more vigorous development of new xylem. Flower buds, on the other hand, initiate a very vigorous and pronounced starch disappearance from the stems, while new xylem formation is very tardy.

The drain upon the starch reserves of a spur and of a whole tree caused by flower bearing is quite surprising. When flowering is followed by "setting" and the development of a fruit, even if it is only to the "June drop" period, the drain upon starch and carbohydrate reserves generally is very much increased. Looked at in terms of total tree performance, a heavy blossoming makes a very heavy demand upon carbohydrate reserves, and at the end of the blooming period the supply is very seriously depleted. This is quite apart from whether the blossoms "set" or not. The extent of the depletion of carbohydrates by excessive blossoming is, I think, not generally realised.

It was indicated earlier that the main objective at the moment in our work is the establishment of diagnostic growth features, so that we can use these as a guide to practice.

In a study of the leaf area of biennial bearing apple trees a few years ago, the writer had occasion to outline and measure as a consecutive series the leaves developed on spurs and current year's extension shoots. In both cases it was found that the leaves passed through a regular change in shape from the oldest to the youngest. It was found that the leaves passed from a rounded leaf at the base to an elongated spearhead-shaped leaf at the tip. This change in shape was not understood for some time. The clue to its meaning was given by some unpublished work of Dr Pearsall of Leeds University, who was investigating the effect of nitrogen supply upon leaf form in *Acer*. He showed that plants grown under conditions of high nitrogen supply had rounded leaves, whereas under conditions of low nitrogen supply the leaves were long and narrow.

The leaf shapes shown by Dr Pearsall correspond to those found at the base and tip of the current year's growth in apple trees at Long Ashton. The investigations into the seasonal cycle of starch in apple trees reported in this paper show that the last formed leaves of the year are produced under conditions of high carbohydrate in the stems. In view of the established relation between carbohydrate and nitrogen in plant tissues, it may be safely assumed that these last formed leaves are produced under conditions of low nitrogen. Their leaf shape confirms this assumption. During the past two years careful observations have been made. Trees which we have every reason to suspect are "high carbohydrate, low nitrogen" trees have long narrow leaves *for the particular variety*, while "high nitrogen" trees have decidedly rounded leaves. In the matter of nitrogen supply, therefore, we have here a diagnostic feature of considerable value and importance.

Applied plant physiology is a promising field for study, and is capable of giving data from which diagnostic features in plant nutrition may be developed, particularly where the work may be carried out simultaneously with chemical investigations in the laboratory. What we need urgently at the moment is more attention paid to the recording and qualitative analysis of the kind of growth that accompanies a given set of environmental conditions, and the correlation of these with chemical data. It is only by a closer correlation of internal conditions with external growth characters that we can hope to direct the growth activity of fruit trees towards the most desirable ends.

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REVIEWS

The Oligochaeta. By J. STEPHENSON, M.B., D.Sc., formerly Professor of Zoology in Government College, Lahore, and in the University of the Panjab, and Lecturer in Zoology in the University of Edinburgh. Pp. xvi + 978, with 242 illustrations. Oxford: Clarendon Press, 1930. 60*s*. net.

Dr Stephenson, who has for the last twenty-three years been a most active investigator of the Oligochaeta, came to the preparation of this work with a thorough grasp of the practical side of his subject—for he has examined some thousands of specimens—and with an intimate knowledge of the literature of the Oligochaeta, and the result is a masterly monograph from the same Press which thirty-five years ago issued Beddard's *Monograph of the Order Oligochaeta*.

The book is composed of twenty chapters, a bibliography, a subject index and a systematic index.

The opening chapter gives a brief account of the external features, beginning with the size—some of the aquatic forms, species of *Aeolosoma* and *Chaetogaster*, are not more than a millimetre long, but examples of the giant earthworm (*Megascolides australis*) of Gippsland have recently been recorded by Mr Charles Barrett that were nine and even eleven feet long when alive. The various forms of prostomium, the segmentation, external apertures, clitellum, etc., are described.

The second chapter is devoted to a consideration of the body wall—the epidermis and its different types of cells, the setae and their glands, the details of structure and arrangement of the musculature, the connective tissue in the cells of which are the problematical bacteroids, and the pigmentation. This leads to an account of the coelom and its subdivisions, the various peritoneal organs and the coelomic corpuscles. Careful attention is given to the functions of the chloragogen cells; it is generally agreed these cells abstract waste matter from the blood, and the evidence that they also act as storehouses for nutrient reserves is presented.

The comparative account of the alimentary canal is a particularly good piece of work. Here the reader will find a critical summary of the various views upon the structure, relations and functions of the chromophil cells, the peptonephridia, and the calciferous glands, which are held to be folds of the oesophageal epithelium and therefore not of mesodermal origin. Attention is directed to the atrophy of the alimentary tract in sexual examples of certain Naididae; such atrophy is not due to pressure of the genital products, as an examination of sections shows, and it occurs over a more extensive region than that occupied by the genital organs. The author suggests that, as the attainment of sexual maturity in these species marks the end of the individual's life, the material of the alimentary canal, being no longer of use, is in some way brought into solution and added to the reserves of the ova.

The following chapter on the vascular system contains an account of the anatomy of the vessels in *Lumbricus* followed by a comparative consideration of each group of vessels in the Oligochaeta, and of their histology. In an interesting section on the evolution of the vascular system the author outlines the views of Lang (the trophocoel theory), that the main vessels are specialisations of a common perienteric sinus and that their positions are determined by the places in which the neighbouring walls of the coelomic chambers come together, and then points out how Vejdovsky's conclusions differ from Lang's. The stages of evolution are illustrated in many of the less specialised Oligochaeta, *e.g.* in *Aeolosoma* the intestinal network (sinus) is in bulk as well as in physiological importance the chief part of the system.

The chapter on respiration gives a clear account of the mechanisms of respiratory interchange through the body wall—which may or may not have networks or loops of blood vessels—or by the agency of gills which are present in half a dozen genera, and describes the phenomenon of intestinal respiration which is met with in certain aquatic Oligochaeta (Naididae, Tubificidae, *Aeolosoma*).

The account of the excretory system is prefaced by a description of the anatomy and histology of the nephridium of the Lumbricidae. The half dozen main types of nephridia found in the Oligochaeta are then considered, and there follow a systematic account of the nephridia in the several families, a résumé of the processes of excretion and a brief note on the evolution of the nephridial system within the Oligochaeta. The whole chapter is a masterly exposition of a difficult subject.

The following chapter on the nervous system includes accounts of its anatomy and histology, the relations of the different neurones to each other, the giant nerve cells and fibres, the physiology of the nervous system in relation to the various modes of locomotion, and a short note on the psychology of earthworms. The next chapter is devoted to the sense organs among which are the photo-receptor cells.

The longest chapter in the anatomical part is one of 115 pages on the gonads, their ducts and associated glands, the spermathecae, the spermatophores, the modified genital setae and the clitellum. This is by far the best comparative account available of the complex reproductive apparatus of this Order. The following chapter deals with spermatogenesis, oogenesis, fertilisation, copulation and oviposition.

In Chapters XII and XIII the author has given a critical digest of present knowledge of the embryology and of asexual reproduction. In Chapter XIV are recorded the chief anomalies of structure and malformations which have been met with, *e.g.* bifurcation of one or both ends, and the next chapter summarises the mass of literature on regeneration and transplantation—for the earthworms have been extensively employed in experimental investigations on these subjects. The author refers to the bearing of some of the results on the germ-layer theory; for instance, that in earthworms there appears to be a difference between the ontogenetic and regenerative processes, the pharynx being of ectodermal origin in ontogeny but endodermal in regeneration.

The chapter on the oecology and manner of life of the Oligochaeta contains a selection of the most interesting observations on these subjects by the writers of the last thirty years. One of the striking references is to the very small oxygen requirements of some of the aquatic Oligochaeta—Tubificids are abundant in the Thames mud below London, and a species of *Limnodrilus* occurs in Peoria Lake (near Chicago), which contains a large amount of organic matter from the packing factories, where the dissolved oxygen was under one part in a million. Reference is made to the castings voided by earthworms, and to the fact that the aperture of the same burrow is used for this purpose for a considerable time and hence castings of large size are formed. The largest found by Darwin at Down weighed nearly 4 oz.; a casting of *Notoscolex birmanicus* in Burma is recorded as being 150 mm. high and weighing $3\frac{1}{4}$ lb. The number of earthworms in the soil appears to be considerably greater than the figure given by Darwin—quoting Hensen—53,767 per acre, "but this figure—less than 30 per square metre—seems to be very small." There is a mistake here—the number stated would be equivalent to less than 14 per square metre. Bretscher, who made observations on various kinds of ground near Zürich, found in a garden 300 Lumbricidae per square metre, in a fir wood 120, in a meadow 700, and in an orchard 720. The Enchytraeids in the same places were respectively 5000, 8000, 8000 and 1650 per square metre.

The author records a number of instances where Enchytraeids were found in damaged roots of celery, cabbage, strawberrry, and in tubers and bulbs, but the worms were probably not the primary cause of the damage, indeed they appear in some cases to bring about the destruction of some of the Nematodes which attack roots of field and garden plants.

The section on the parasites of Oligochaeta includes an account of Dr Keilin's work on the parasitisation of *Allolobophora chlorotica* by the larvae of the cluster fly—*Pollenia rudis*—and the statement is added on the authority of Prof. Cockerell that this fly has become abundant in Colorado since European earthworms were brought in with plants, and in places is now a veritable plague.

In the excellent chapter on geographical distribution are set forth the arguments of Michaelsen and of Benham, regarding the value of the evidence afforded by the distribution of the Acanthodriline worms concerning the former existence of a more extensive antarctic continent. The author adds his own careful deductions on the bearing of the facts of geographical distribution on the former existence of Indo-Australian and other land-bridges.

The discussion of the phylogeny and affinities of Oligochaeta is interesting for the views expressed on the close relationship of Oligochaeta and leeches, on convergence and on polyphyly.

The systematic chapter—a couple of hundred pages—opens with a scheme of classification into fourteen families for each of which is given a concise definition, with a note of the distribution and observations on the chief structural features. The author has restricted the further systematic consideration to the genera—about 120—for each of which a definition, reference to its distribution and a note of the number of species are given. The number of species of Oligochaeta now known is about 2400, twice as many as were known in 1900, and a critical revision of them was not possible. The author makes a strong plea in the preface for careful identification of specimens employed in research. As he points out, it is useless to refer to a specimen as "the earthworm" for in Britain there are nearly forty species of earthworms belonging to eight genera. The expression "the common earthworm" is meaningless, for what is the common species in one locality is not so in another, and "the common earthworm, *Lumbricus terrestris*" is for many parts of the country fallacious. The author states that in his experience of earthworms from the Edinburgh area, *L. terrestris* stands third in order of frequency, being preceded by two species of *Allolobophora*—a fact which the reviewer confirms as the result of some twenty years' experience in the collection and examination of species for class work.

The bibliography of more than 1000 papers includes the more important works down to October 1928 and a few more recently issued. Only about 80 bear date previous to 1895, the author rightly considering that the papers of an earlier date had been dealt with by Beddard or that the subjects of which they treat had been re-investigated by newer methods.

The 242 illustrations have been carefully chosen and are well reproduced, and nearly all are from recent memoirs.

The book appears to be entirely free from misprints—a testimony to the meticulous care of the author.

The preparation of this monograph represents a large amount of detailed work and critical consideration and is altogether a fine achievement on which the author is to be most heartily congratulated. The volume will undoubtedly be the authoritative work of reference on the Order for many years; it is eminently satisfactory alike in its treatment of detail and in its breadth of view.

J. H. ASHWORTH.

Organic Chemistry in Biology and Medicine. By GEORGE BARGER. McGraw-Hill Publishing Company, Limited.

In these days of specialisation, when organic chemistry is no longer merely the chemistry of organic substances, it is refreshing to read a book by one who, although primarily an organic chemist, has invariably given the biological applications of his subject the attention which they deserve. A book such as this will do much to prevent the widening of the gap between organic chemistry and the biological sciences.

The choice of subjects is a particularly happy one. Chapters on the chemistry of the hormones and vitamins, chemical constitution and physiological action, and chemotherapy will be of equal interest to the chemist and the physiologist. The chapter on the hormones is perhaps somewhat unbalanced. Apart from a short introduction, this section deals exclusively with adrenaline and thyroxine. A short account of some of the recent chemical work on insulin would not have been out of place. In the chapter on chemical constitution and physiological action, the author provides an

excellent summarised account of much of his own classic work on the natural bases. Such a chapter, as well as the following one on chemotherapy, would be welcome additions to many of the somewhat incomplete textbooks of biochemistry.

Not the least of the many attractions of this volume is the author's easy style of writing which makes its perusal a pleasure rather than a labour. Being based upon a series of lectures delivered at Cornell University, the cold impersonal atmosphere of the average scientific textbook is replaced by the more personal atmosphere of the lecture room.

G. F. MARRIAN.

Studies of the Sirex Parasites. By R. N. CHRYSTAL, Hon. M.A., D.Sc. (Edin.). Oxford Forestry Memoirs, No. 11, 1930. Pp. 63, with 10 plates and 7 text-figures. Oxford: at the Clarendon Press. Price 5*s*.

This well-produced memoir is the outcome of investigations made, in the first instance, in co-operation with the Parasite Laboratory of the Imperial Bureau of Entomology, at Farnham Royal. The object was to study the parasites of wood-wasps (*Sirex*), and more particularly the Ichneumon *Rhyssa persuasoria*. In conjunction with Dr J. G. Myers the latter parasite and its *Sirex* host were investigated as completely as possible, with the intention of applying the knowledge so gained to the biological control of *Sirex* in plantations in New Zealand. The result has been to ship large numbers of the *Rhyssa* to that country, in order to attempt their colonisation in areas affected by the wood-wasps. A second species of parasite, namely *Ibalia leucospoides*, also attacks the *Sirex*, and the present memoir is largely based upon researches carried out on this insect at the Forestry Institute at Oxford, during the past three years. Until this study was undertaken little was known of the biology of any species of *Ibalia*, and for the most part they have been regarded as rare insects. Dr Chrystal, however, has discovered the species *I. leucospoides* in comparative abundance in certain suitable English localities, frequented by its host *Sirex cyaneus*, and obtained sufficient material to investigate its morphology and biology to a very full degree. His account forms one of the most complete studies available of any individual species of Hymenopterous parasite, and the memoir for this reason is one of special interest to all entomologists interested in problems of biological control.

It appears that the *Ibalia* oviposits in the egg-tunnels drilled by the female *Sirex* in solid wood. It inserts its ovipositor deep down until it finds a host larva within which it deposits its egg. As a rule, only a single egg of the *Ibalia* is deposited in each host, but in some cases two or more may occur. The first instar larva is unique among the Cynipoidea in being of the polypod type, with twelve pairs of trunk appendages and a definite caudal prolongation. No other member of the group is known to emerge from the egg in this phase, and the discovery is one of considerable biological interest. It issues surrounded by a trophamnion, and after this membrane has been shed the remains can be easily found among the tissues of the host larva. In its second and later instars the form of the *Ibalia* larva is greatly altered, since the segmental appendages are totally lost, and the caudal prolongation becomes progressively reduced to a small rudiment. The duration of the whole larval period of the parasite extends for the exceptionally long period of three years, and for almost two-thirds of this time it is living within the body of its host.

Dr Chrystal discusses the relationship of *Ibalia* and the *Rhyssa* parasite previously alluded to. He advances evidence which indicates that individual *Sirex*, parasitised by *Ibalia*, are also liable to be parasitised by *Rhyssa*, and for this reason it is believed that when the latter insect is abundant the *Ibalia* will only occur sparingly. Whether it is advisable to introduce *Ibalia*, as well as *Rhyssa*, into New Zealand is doubtful, and more data are necessary before any definite decision is reached upon this important practical proposition. It is only possible in this short notice to call attention to certain interesting points in this memoir, and we congratulate its author on the production of a most painstaking and interesting contribution to our knowledge of parasitic Hymenoptera.

A. D. IMMS.

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VOLUME XVII NOVEMBER, 1930 No. 4

THE TRANSMISSION OF STREAK DISEASE BETWEEN MAIZE, SUGAR CANE AND WILD GRASSES

BY H. H. STOREY, M.A., PH.D.

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(With Plates XL–XLIII.)

CONTENTS.

INTRODUCTION.

THE name of *Streak Disease* has been used to designate a characteristic chlorotic condition which has been observed in South Africa in a number of species of plants of the family Gramineae. The evidence, which has been published in several papers dealing with its occurrence and behaviour in maize (*Zea mays* L.) (11, 13) and in varieties of sugar cane (*Saccharum* spp.) (10), suggests that it belongs to the group of virus diseases. In these papers it was shown that the leafhopper, *Cicadulina* (*Balclutha*) *mbila* Naude (2), was capable of transmitting the disease from affected plants of each species to healthy plants of the same species; that is, that this insect

might transfer the disease from affected maize to healthy maize and from affected cane to healthy cane. Furthermore, as we show in this paper (see Appendix), there is a strong probability that this species is the only vector of streak disease in South Africa.

Upon the evidence up to this point it might well be assumed that a single virus was the cause of streak disease in both maize and sugar cane. We now describe our attempts to transmit the disease from one species to the other, in the course of which the expected results were not obtained. The virus from maize, as usually found in the field, caused only a transitory infection of sugar cane, from which the plant subsequently recovered. The virus from sugar cane produced in maize a permanent but mild form of streak disease. The passage of each virus through the second host plant produced no apparent change in its virulence to the original host plant. We conclude that the virus of maize streak and the virus of cane streak are not identical.

We have studied, furthermore, the transmission of streak disease to and from certain wild grasses. While our results are in some respects puzzling, they again demonstrate the differential effect of the maize and the cane viruses; they suggest, but by no means conclusively, that there may be streak viruses specialised to these hosts.

Finally we have studied the infection by this disease of the cane variety P.O.J. 213, hitherto regarded as immune (10). While even more puzzling complications have been met with in this work, our evidence is perhaps of value as affording insight into the behaviour of a virus in a plant having a very high resistance to its effects.

We had hoped to press these investigations to some more definite conclusion. But a hailstorm of great violence in June 1929 reduced our Durban greenhouses to ruins, and destroyed all experimental plants and insect cultures. The time seemed appropriate, therefore, for a progress report.

These studies have been carried out during the period 1924–9. For those relating to the transmission between maize and Uba cane, H. H. Storey is mainly responsible[1]; while A. P. D. McClean has taken the main part in the later studies of wild grasses and P.O.J. 213. We make acknowledgment to Dr I. B. Pole Evans, Chief of the Division of Plant Industry, Union of South Africa; to Mr R. F. W. Nichols, who has taken an important part in the manipulation of experiments, and to Field Inspectors J. S. Mackay and C. E. Levett for assistance in various stages of the work.

[1] This portion of the work formed a part of a thesis accepted by the University of Cambridge for the degree of Doctor of Philosophy.

Experimental methods.

We have been able to find no method for the transmission of the streak virus except by using *C. mbila* as a vector. Consequently in all the transmission experiments described in this paper this insect has taken an essential part. While the breeding and manipulation of an insect vector have involved much labour and consumed much time, the use of the small leaf-cage method permitted a reasonable replication of experiments within the space at our disposal. Our technique has been described in earlier papers (11, 13). Where a group of leafhoppers was under test, they were confined in a glass tube, about 8 in. long by 1 in. diameter, which was arranged to enclose the tip of a leaf. Single hoppers were usually manipulated in small glass tubes clipped on to the leaf. In certain experiments with grasses it was found convenient to cover the whole plant with a glass lamp chimney, after the manner illustrated by Kunkel (5). All experiments were performed in a greenhouse with gauze protected ventilators, and the precautions against insect infestation, described in an earlier paper (13), were observed.

Plants of maize, of the variety Hickory King, and of the grass species, were raised from seed within the gauzed greenhouse. In all cases an approximately equal number of control plants from the same sowing was retained alongside those under experiment; records of these controls have usually been omitted from this report, except where a control plant became diseased. The cane was raised from setts cut in a field showing a very low proportion of streak-diseased plants, and in all the later work control setts were cut from the same sticks as those which provided the experimental plants.

Streak disease in maize and sugar cane.

The starting point of these studies has been the disease as it occurs in each host in the field. Thus, in the beginning, our maize streak virus was taken from a naturally streaked maize plant; and since our usual procedure was to employ, as the source of infection for each experiment, the plants which had become diseased in an earlier experiment, in the main we were probably using in our earlier work a single strain of virus. When, in the course of our work upon transmission between maize and cane, it became evident that a maize plant might be carrying at one time more than one virus, we adopted the following procedure to ensure the purity of the maize virus with which we were experimenting. Maize seedlings were subjected to infection by hoppers which had obtained the virus

from maize. Now one of us has shown (13) that hoppers obtain the virus readily only from the actual chlorotic areas of the maize leaf; furthermore, we have observed that the period elapsing between the infection of a maize plant and the first appearance of streak signs is shorter with the more virulent virus from maize than with the mild virus from cane. Consequently the first spots to appear in an infected plant were likely to contain only the most virulent virus. On these spots, therefore, new non-infective hoppers were allowed to feed, and these in turn then infected new plants. By repeating this process several times, we hoped to isolate a virus highly virulent to maize, uncontaminated by any other virus less virulent to maize, but possibly more virulent to other species of plants.

Streak virus of cane was obtained from the appropriate variety of cane naturally infected in the field without any special precautions.

The streak virus from maize.

This disease, with its pronounced and characteristic signs of parallel broken chlorotic stripes upon the maize leaf (Plate XL, fig. 2, and Plate XLIII, fig. 12), has been previously described, and its transmission by *C. mbila* demonstrated (11). A large number of experiments (13) has since confirmed the ability of this leafhopper to acquire the virus by feeding on diseased maize and to transmit it to healthy maize plants, with the production of normal symptoms.

Following the glass-tube method of experiment, attempts were made to transmit streak from maize to the Uba variety of sugar cane. In preliminary trials, of twenty-one plants exposed to the feeding of hoppers known to be infective to maize, one became diseased. Since, however, the previous history of the hoppers used in this one experiment was uncertain, and it is possible that they had fed at some time upon diseased cane, the result is not considered to be significant. Later experiments, in which hoppers bred from eggs under controlled conditions were alone used, failed to give any permanent infections of cane. The results given in detail in Table I show that all of the thirty-six plants in four separate experiments resisted permanent infection by the maize virus. One of these experiments, on November 5th, 1925, was run in parallel and under identical conditions with a successful experiment in transmission from cane to cane. (See experiment of November 5th, 1925, in Table II.) The observations recorded in the last column of Table I will be considered later.

Since it was conceivable that the tube technique hindered in some way the transmission from maize to cane, an experiment was started in a large cage arranged to reproduce as nearly as possible the conditions

holding in the field. This cage, about 8 ft. cube, of fine wire gauze, contained forty-eight Uba cane plants and seventy-two maize seedlings. On September 24th, 1925, two streak-diseased maize plants were introduced, bearing many leafhoppers in different stages of development. All the healthy maize seedlings became streak diseased, and after 60 days they were cut off and left to dry among the cane plants. Later, very many hoppers, adult and immature, were to be seen feeding upon every cane plant. On the 75th day, twelve adult hoppers were removed for a test, and of these hoppers five individuals were infective to maize. Meanwhile the cane plants had remained healthy, except that a few had shown one or several streak markings without any later full development of the disease; at the conclusion of the experiment, 240 days from the start, all cane plants bore young leaves which were entirely free from streak signs. More severe conditions for streak infection than this experiment afforded could hardly be conceived. Furthermore, a later experiment showed that the experimental technique was indeed favourable to streak infection of cane, provided that a suitably virulent virus was available. Here the source of infection was diseased Uba cane; within 4 months streak had appeared in two out of four healthy Uba plants, in four out of five plants of the cane variety CH 64/21 and in all of seven plants of the variety Merthi.

Table I.

The maize virus—Transmission to cane.

Hoppers, in different stages of development, bred on diseased maize, fed for 12–30 days in glass tubes on leaves of cane plants.

Date	No. of hoppers in each tube	Period of observation (days)	No. of plants tested	No. of plants permanently infected	Remarks
6. iv. 25	4	65	6	Nil	
5. xi. 25	4	61	12	Nil	2 developed a few streaks 5 doubtful streaks
	Controls	61	12	Nil	No streaks developed
6. v. 26	12	100	14	Nil	4 developed streaks 1 plant which developed a few streaks (from which the disease was transferred to maize) was kept under observation for 220 days and was still healthy
	Controls	100	15	Nil	No streaks developed
28. vii. 27	50 to 100	340	4	Nil	3 developed a few streaks. Later 2 cut back and allowed to ratoon. No recurrence of streaking at any stage
	Controls	340	4	Nil	No streaks developed

Similar failures were encountered in an experiment in the transmission of maize streak to Uba cane which was growing under normal conditions in the field[1]. Tubes, each containing twelve hoppers bred on diseased maize, were placed on leaves of Uba cane plants in a field which was almost free from streak disease. Of eight plants so treated all remained healthy and ratoons from the same stools were still healthy 15 months later. In a parallel experiment, hoppers which had been bred on diseased cane infected three plants out of eight. Adjacent uninoculated plants remained healthy during the period of observation.

During the course of the experiments in transfer of the maize virus to Uba cane, we frequently observed in the young leaves of the cane plants one or rarely several large isolated chlorotic spots or streaks (see the last column of Table I). Plate XL, fig. 1, is from a photograph of a typical streak in a leaf of a Uba plant from one of these experiments. Usually these streaks appeared in 2 to 3 weeks after exposure to infection; and subsequently healthy leaves only were produced up to the end of the period of observation, varying from 61 to 340 days. No control plant ever showed any leaf markings of the kind seen in the inoculated plants.

The presence of the virus within these chlorotic areas was demonstrated by feeding uninfective hoppers upon the areas. Of twenty-one hoppers so fed and subsequently tested individually upon maize seedlings, seven produced the typical maize disease. Attempts to infect cane plants by means of three survivors of the above seven infective hoppers failed. Hoppers, bred upon a maize seedling which had been infected by one of the above seven hoppers, failed, when in groups of sixteen, to infect either of two cane plants. These experiments, therefore, give no indication that the virulence of the maize virus to cane had been enhanced by passage through cane.

We have not overlooked the possibility that these plants, which had shown an apparently transitory infection, might nevertheless continue to harbour the virus, and might at some later stage of growth develop streak disease in its full manifestation. Indeed in the P.O.J. 213 variety of sugar cane we observed a recurrence of streak disease in a plant which we had supposed to have recovered, as will be described hereafter. For this reason we retained in the greenhouse the plants of one experiment for as long as 340 days, and some of these plants were during this period cut back and allowed to ratoon. Nevertheless, no recurrence of streak signs was ever observed.

[1] At the Sugar Experiment Station, Mt Edgecombe, Natal, by kind permission of the Director.

Furthermore, we have attempted to determine whether the virus of maize streak may continue to exist in these cane plants which have recovered. A large number of non-infective hoppers was fed for 30 days on a Uba plant which had developed one clear streak in an experiment shown in Table I; this group of hoppers subsequently failed to infect either of two maize plants upon which it fed in successive tests. This evidence, however, is not conclusive. For if the virus was indeed present in the plant it is improbable that it would be in a form, or in a position, where it might be taken up by feeding hoppers; since one of us has shown (13) that, in maize streak, hoppers only rarely, if ever, obtain virus from the green parts even of a fully streaked leaf.

The streak virus from sugar cane.

The disease in the Uba variety. The appearance of streak disease in Uba cane as it occurs in the field in Natal has been already described (10). The symptoms generally resemble those of streak in maize except that the chlorotic areas are narrower and more sparsely distributed upon the leaf surface (Plate XL, fig. 4).

An earlier paper (10) described preliminary experiments in which infection of five Uba cane plants out of nine resulted from the feeding individually of leafhoppers of the species *C. mbila*, which had been collected upon diseased cane in the field. Subsequent experiments confirmed this result, seven positives resulting in twenty-seven trials.

Similar positive results were obtained when hoppers, which had been bred from the egg and allowed to feed for varying periods on diseased Uba cane plants, were tested upon healthy cane plants. Groups of four to six of these hoppers infected fourteen out of twenty-two plants (Table II). In all these experiments the first chlorotic streaks appeared after 2 to 3 weeks; thereafter the frequency of streaking increased as new leaves unfolded, until the young leaves were indistinguishable from those of a naturally streaked plant. Frequently secondary shoots appeared also fully streaked.

Experiments under field conditions in which hoppers carrying the cane virus infected three out of eight cane plants have already been noted.

When hoppers, which were capable of infecting sugar cane, were allowed to feed upon maize, they usually produced in the maize a disease resembling in its signs streak in cane; that is, the chlorotic areas were narrower and more sparsely distributed than in a normal maize streak infection. The difference was pronounced and admitted of no confusion

(Plate XL, figs. 2, 3). Nevertheless, the chlorotic areas differed only in size and were otherwise similar (Plate XLIII, figs. 12, 13).

Table II.

The cane virus—Transmission to cane.

Hoppers, either hatched and reared on diseased cane, or bred on healthy maize and fed for a period on diseased Uba cane. Then fed for 12–14 days in groups by glass-tube method on leaves of healthy Uba cane plants.

Date	No. of hoppers in each tube	Period of observation (days)	No. of plants tested	No. of plants infected
5. xi. 25	4	61	12	7
	Controls	61	12	Nil
23. ix. 26	6	35	6	5
	Controls	35	3	Nil
14. ii. 29	6	81	4	2
	Controls	81	4	Nil

We have satisfied ourselves that the differences here observed have not been due merely to constitutional differences in the individual plants used in the experiments for the differences in manifestation in the infected maize seedlings have been consistently maintained during transmission by leafhoppers. One hopper, originally collected upon diseased cane, lived for 5 months in our experiments, being transferred to four cane plants, of which two became diseased, and to five maize plants, of which three became diseased. The signs in all three maize plants were of the sparse type. Of forty hoppers, bred from the egg on streak-diseased Uba cane, six infected maize seedlings when tested singly. The disease in all six plants was of the sparse type. Eleven hoppers, collected upon diseased cane in the field, which had caused infection of cane in the experiments already mentioned were tested on maize; nine gave sparse infections and two the severe maize type of infection. These two hoppers, however, might have fed at some time upon streak-diseased maize which was growing alongside the cane; for, as we shall show later, it is possible for an insect to carry both viruses simultaneously.

As a maize plant infected with the cane virus grew, the new leaves produced bore progressively more sparse streaking until frequently the youngest would bear but few streaks. In no instance, however, could it be said that the plant within the period of observation allowed by a short-lived annual had made a recovery from the disease. A similar tendency towards suppression of symptoms has been observed exceptionally in maize affected with the maize virus; but in the most pronounced case observed, the resulting sparseness of the streaking did not reach that of a cane virus infection, nor were the streaks of the same narrow width.

The signs of the cane virus in maize are often so insignificant that they would escape notice normally in the field. We have, however, observed this sparsely streaked condition in maize planted alongside streak-diseased Uba cane.

The incubation of the cane virus was longer in the maize plant than that of the maize virus under similar conditions. In parallel experiments, the first signs produced by the maize virus appeared on the 8th day, while those of the cane virus appeared in two plants in 12 days and the average for six plants was 17 days.

The passage of the cane virus through maize has, in our experiments, produced no increase in its virulence to maize. In a serial experiment the cane virus was passed through six series of maize plants, the most "severely" affected plant of each series being used as the source of infection for the next series (Table III). The later series showed no more severe symptoms on the average than the second series (Plate XL, fig. 3).

Table III.

The cane virus—Serial transmission through maize.

In each series (except the first) a number of uninfective hoppers were fed for about 7 days upon the relative plant acting as source of infection. Hoppers then tested singly on maize seedlings.

Series	Source of infection	No. of hoppers tested singly on maize	No. of maize plants infected
1st	Diseased cane in field (hopper collected)	1	1
2nd 19. v. 25	Diseased maize plant in 1st series	7	4
3rd 11. vii. 25	Most "severe" plant of 2nd series	9	5
4th 4. ix. 25	Most "severe" plant of 3rd series	12	6*
5th 20. x. 25	Most "severe" plant of 4th series	11	5
6th 14. xii. 25	Most "severe" plant of 5th series	9	4

* One control plant became diseased at a late stage in this experiment.

In the passage of the cane virus through maize its virulence to cane is not lost. Hoppers were fed for 26 days upon a maize plant, which had been experimentally infected from Uba cane and was showing the typical sparse signs. These hoppers then fed in groups of five upon ten Uba cane plants, of which six developed streak disease. Eight control cane plants remained all healthy.

The infection of a maize plant with the cane virus does not hinder its infection later with the maize virus. A maize plant, infected from cane

and showing the typical sparse disease, was subjected to the feeding of a hopper bearing the maize virus. In the expected period the large streaks of a normal maize infection began to appear upon young leaves which were already bearing the fine streaks of the cane disease. At a later stage the young leaves bore the full signs of the maize disease, masking completely the signs of the previous infection by the cane virus. Plate XLI, fig. 6, depicts successive alternate leaves from this plant, showing the lower leaf uniformly sparsely streaked, the transition zone, and the upper leaf normally fully streaked.

A hopper carrying the cane virus was able to take up the maize virus also. In an experiment, a hopper, after feeding upon diseased cane, produced the sparse form of disease in a maize seedling upon which it had fed. It was then placed on a normally streaked maize plant for 4 days and re-tested on a second healthy maize seedling; this seedling developed the normal severe form of maize streak. Similar results were obtained with two hoppers collected upon diseased cane, which after first infecting maize with the sparse disease, later, following a period of feeding on fully streaked maize, infected maize with the normal severe disease.

Reference may here be made to certain experiments with those maize plants in which streak signs, after a normal full development following infection with the maize virus, gradually became sparse during the later growth of the plants. These experiments, however, failed to demonstrate any permanent attenuation of the virus. From two plants in which the streaking became sparse, twenty-one maize plants were infected; these plants showed a slight tendency to become sparse in late growth. From the most sparse plants of this series the virus was transmitted to eleven more healthy plants. Two months after infection these plants were noted as exhibiting normal full signs of maize streak.

The disease in the variety P.O.J. 213. Following upon observation of this variety from 1922–6 in many localities in Natal, where frequently the streak infection in adjacent Uba cane was severe, one of us reported it to be probably immune (10). In January 1926, however, we discovered a plant with the clear signs of streak disease[1]. The lower leaves of this plant bore streaks at a frequency rather less than that normal in Uba, while upon the younger leaves the frequency was very sparse. Three months later the majority of the shoots of this stool bore young leaves which were entirely healthy, a few only having an occasional streak. This observation led one of us to think that this plant had gone through a

[1] At the experiment station of Messrs African Explosives and Industries, Ltd., whose assistance in these observations we acknowledge.

transitory infection of the kind which we have encountered in Uba cane inoculated with the maize virus (12). This was, however, incorrect, for the disease gradually reasserted itself and, by December 1926, many shoots from this stool were fully streaked. During the years 1927–9 the stool continued to produce some healthy and some diseased shoots. Sometimes the distribution of the streaks on the leaves was sparse and irregular, sometimes uniform and at a frequency rather lower than in the normal Uba leaf. Generally the streaks were wider than those in Uba; Plate XL, fig. 4, illustrates a normal fully diseased P.O.J. 213 leaf and a diseased Uba leaf for comparison.

Meanwhile two more diseased stools appeared in 1928 in this cane plot, and six in 1929 in an adjacent plot. These diseased plants were not grouped but scattered through the plots.

Cuttings from these diseased stools, when planted in Durban, produced sometimes healthy and sometimes diseased plants. Detailed notes of these plantings follow; it will be observed that some cuttings from shoots which were unquestionably streaked produced nothing but healthy shoots; others produced shoots which were at first streaked but later recovered and showed no recurrence of the disease.

Cuttings taken from the original streaked stool of P.O.J. 213.

(i) 13. xii. 26. Cuttings taken from shoots showing streaks on leaves. Planted in greenhouse. Produced healthy plants which remained free from streak signs for one year.

(ii) 29. vii. 27. Cuttings of shoots, showing streaks on leaves, planted in open. First four leaves of all shoots arising from these cuttings all healthy. Later streak began to appear in some mother-shoots and in secondary shoots arising from them. Other shoots, arising from buds on the same setts, were healthy. By January 1st, 1928, one sett producing shoots all streaked (rather sparse frequency). Another sett—middle bud producing streaked shoots (rather sparse), upper and lower buds producing healthy shoots. On March 28th, 1928, all shoots streak-free. Cut down. Ratoon shoots at no stage produced streak leaves, and on May 1st, 1929, all shoots were entirely healthy. Cuttings from these ratooned plants produced only healthy shoots.

(iii) 21. x. 27. Cuttings from the streak-free shoots, planted in open, produced shoots entirely healthy up to May 1st, 1929.

Cuttings from streaked shoots, planted in open, produced shoots mostly healthy; a few more or less sparsely streaked. Several cuttings produced both healthy and diseased shoots, with a tendency for streak to occur in the buds along one side of the sett only, but not invariably. On August 23rd, 1928, all shoots bearing only healthy leaves. Cut down. Ratoons all healthy up to May 1st, 1929, except that one shoot showed two streak-like markings. On November 3rd, 1929, all plants entirely healthy. Cuttings taken from these plants produced only healthy plants.

Cuttings taken from another stool fully streaked.

(iv) 10. i. 28. Two cuttings planted in open. Cuttings produced only healthy shoots. On August 23rd, 1928, cut back. Ratoons healthy up to November 3rd, 1929.

(v) 15. x. 28. Two setts planted in open. Resulting shoots mostly showed streak, varying from one spot to a medium uniform frequency. By May 1st, 1929, all shoots from one sett healthy; a few streaks only on one or two shoots of other plant. On June 22nd, 1929, taken into greenhouse. The shoots of the one stool remained healthy. In the other stool frequency of streaking gradually increased until by November 3rd, 1929, all shoots were normally streaked.

(vi) 25. i. 29. Four setts planted, two in greenhouse and two in open. Setts in greenhouse produced shoots all streaked and not losing streaking up to November 3rd, 1929, setts in open more sparsely streaked and tending to become more sparse. Later streak increased and by November 3rd, 1929, all shoots were normally streaked.

These observations are difficult to interpret and the reason for their diversity is obscure. They plainly show, however, the instability of the virus infection in this cane variety. Transferred to a new locality diseased plants in the main recovered (temporarily, at least) from the disease. A somewhat similar instability in the mosaic disease in certain cane varieties has been reported (*e.g.* by Stahl and Faris (9)).

The naturally streaked plants of P.O.J. 213 were discovered in a plot growing at Umbogintwini, Natal, in a situation where experience had shown the streak infection in Uba cane to be very severe. Some years previously a plot planted with healthy Uba setts had become entirely diseased within the first 9 months of its growth. This plot was still growing, fully diseased, alongside the P.O.J. 213. Consequently it was probable that the P.O.J. 213 was visited by many hoppers carrying the Uba cane virus. The indication is, therefore, that the P.O.J. 213 variety became infected with the virus from Uba.

Experiments in transmission from P.O.J. 213 support this idea. Hoppers which had fed on streaked P.O.J. 213 were subsequently fed upon healthy maize seedlings. In six separate experiments, ten plants became diseased out of a total of sixty-one plants tested. All the diseased plants showed the sparse form of the disease typical of that caused by the Uba cane virus (Plate XLII, fig. 8). New hoppers fed upon one of these diseased plants infected three out of nineteen maize plants with the sparse form of the disease and one out of twelve Uba cane plants with the normal Uba form of the disease.

Five separate experiments in transmission from P.O.J. 213 to Uba cane resulted in twelve infections of forty-four plants tested. The signs of the disease in these experimental plants were not distinguishable from those produced in similar plants by the virus from Uba cane (Plate XL, fig. 5).

Success in experimental transmission to P.O.J. 213, in view of the rarity of its occurrence in the field, was hardly to be expected. Our first eight experiments in transmission from P.O.J. 213 to P.O.J. 213, in-

volving 109 plants, were all failures. Two experiments designed to transmit streak from Uba to sixteen plants in all of P.O.J. 213 both failed. Five plants of P.O.J. 213 remained healthy when fed upon hoppers carrying the virus from a Uba cane plant which had been experimentally infected with the virus from P.O.J. 213. A large number of experiments, in which hoppers carrying the maize virus fed upon healthy P.O.J. 213 plants, gave no result. Nor did any infection result in fifteen plants from the feeding simultaneously on each plant of hoppers carrying both the maize and the Uba viruses.

Eventually, however, in the experiments detailed in Table IV, we were successful in infecting four plants of P.O.J. 213; three plants from P.O.J. 213 and one plant from Uba. The infection of these plants appeared to be permanent and we observed no tendency to recovery. It is not clear what reason accounts for these successes after so many failures. Conceivably we were fortunate in choosing setts from stools which had for some reason a lower resistance than usual. We have not overlooked the possibility that, in spite of our precautions to secure truly representative controls (see heading to Table IV), our experimental plants may have been carrying the virus from an old infection in the field. But in the experiments already described we found that cuttings of apparently healthy stalks, even from a stool partly diseased, produced only healthy plants. Furthermore, the appearance of the disease in four inoculated plants out of seventeen, as compared with seventeen controls which remained healthy, does not favour a chance explanation.

Table IV.

The cane virus—Transmission to P.O.J. 213.

Hoppers, bred on healthy maize, fed for 20 or more days on streaked P.O.J. 213 or streaked Uba cane. Caged in glass tubes in groups of six on single leaves of healthy P.O.J. 213 plants. Setts for experimental P.O.J. 213 plants cut from stools not known ever to have shown streak. Control setts cut from same stalks as those inoculated. In experiment of 28. v. 29 each stalk cut into four pieces, the top and bottom acting as controls and the two middle pieces being inoculated.

Date	Source of virus	Period of observation (days)	No. of plants tested	No. of plants diseased
25. iii. 29	Streaked P.O.J. 213	82	5	2
	Controls	82	5	Nil
25. iii. 29	Streaked Uba	82	4	1
	Controls	82	–	Nil
28. v. 29	Streaked P.O.J. 213	83	8	1
	Controls	83	8	Nil

The disease in other cane varieties. An earlier paper (10) recorded the names of eleven varieties of sugar cane which had been observed in Natal

bearing the typical signs of streak disease. To this list may now be added the following varieties:

Variety	Origin	History of the sample tested
Black Tanna (Plate XLII, fig. 9)	Old standard variety	An early importation, probably from Mauritius
M.P. 55	A Mauritius seedling	Ditto
CH 64/21	Reported to be a cross between Uba and D. 74	Received from Experiment Station, Chaparra, Cuba
Merthi	Thin cane of the Uba type	Received from U.S. Dept. Agric. Washington
Oshima	,,	,,
Kavangire	,,	,,

An experiment has been carried out with the variety M.P. 55. Hoppers fed on a diseased plant of this variety transmitted streak to two out of ten maize seedlings, the manifestation of the disease being typical of that produced by the virus of Uba cane.

Certain experiments, following the large cage method, have been performed in transmission of streak from Uba cane to a number of varieties recently imported to Natal. These experiments have led to the following classification:

Variety	Origin	History of the sample tested
CH 64/21	Reported to be a cross between Uba and D. 74	Received from Experiment Station, Chaparra, Cuba
Merthi	Thin cane of the Uba type	Received from U.S. Dept. Agric. Washington
Kavangire	,,	Ditto
Zwinga	,,	Received from Tucuman Experiment Station, Argentina (through Incomati Estates, Portuguese East Africa)

Variety	Provisionally regarded as immune origin	History of the sample tested
Co 205	Seedling raised at Coimbatore, India	Received from Coimbatore
Co 210	,,	,,
Co 213	,,	,,
Co 214	,,	,,
Toledo	Local variety—selected in Philippine Islands	Received from U.S. Dept. Agric. Washington
Hinde's Special	,,	Received from Philippine Sugar Association
Kassoer	Believed natural cross between Cheribon and *Saccharum spontaneum*	Received from U.S. Dept. Agric. Washington
Kinar	Thin cane of the Uba type	Ditto

Streak disease in wild grasses.

We have observed a number of species of grasses in the field bearing chlorotic leaf-markings resembling those of streak disease in maize and sugar cane. A list of sixteen species was published in an earlier paper (10). To this list we are now able to add the following names:

Name	Locality
Dactyloctenium aegyptiacum, Willd.	South Africa
Tragus racemosus, Scop.	,,
Setaria verticillata, Beauv.	,,
Rottboellia exaltata, L.f.	Usambara, Tanganyika Territory
Cymbopogon citratus Stapf.	Uganda
Diplachne eleusine Nees.	South Africa

The diagnosis of the disease in all these species is based upon the signs observed in plants in the field. But the majority of the species in the two lists showed markings so characteristic of streak disease, as it may be seen in the hosts which we have studied, that diagnosis is reasonably secure. Where the markings were not fully typical, plants were transplanted and maintained under observation. The study of these plants showed that the disease was both permanent and systemic in them. Caution is however necessary in drawing conclusions from the preceding evidence, for we have not transferred streak disease experimentally to or from any of the grass species, except *Digitaria horizontalis* and *Eleusine indica*. With some species the manner of transmission is obscure, for they appear to be unfavourable food plants for *Cicadulina mbila* and unable to maintain hoppers alive for more than a few days.

The disease in Digitaria horizontalis *Willd.*

This common ruderal species is the one that may be most frequently seen streak diseased in Natal. The signs of the disease show considerable variations in different individual plants. Plate XLIII, fig. 14, illustrates a typical diseased plant, and Plate XLII, fig. 10, separate leaves of diseased plants.

We have found diseased *D. horizontalis* in many localities but particularly in maize fields in which the maize is reaching maturity. Indeed it has been a common experience for us to find the weed flora of lands from which the maize has been recently reaped to consist almost entirely of streak-diseased plants of this species. During the 1926–7 season boxes of young seedlings of *D. horizontalis* were exposed each month from August to March in a maize plot where streak disease was severe. A

proportion of each month's planting became diseased. The field evidence, therefore, suggests that the maize virus is capable of infecting *D. horizontalis*.

Experiments have confirmed the ability of the maize virus to infect this grass species. Hoppers, which had been bred upon streaked maize, were caged separately in groups of six on twelve *Digitaria* plants, all of which became streak diseased. Of the twelve control plants, one (upon which an escaped hopper was caught) also became diseased. From these infected *Digitaria* plants the virus was re-transmitted to maize by uninfective hoppers, which, after feeding on a diseased *Digitaria* plant, infected twelve out of sixteen maize seedlings. The disease in these maize seedlings was of the normal maize type. Transmission from maize to *D. horizontalis* was also obtained under Tanganyika conditions. Using as a source of the virus diseased maize collected in the vicinity of Amani, three plants of *Digitaria* were infected out of six. The signs of the disease in these plants were similar to those observed in this species in Natal.

We failed to transfer the virus from sugar cane to *D. horizontalis*. Groups of five hoppers each reared upon streaked Uba cane were tested on five *Digitaria* plants, which remained healthy. The same hoppers subsequently infected maize seedlings with the sparse form of streak, showing that they were undoubtedly carrying the cane virus during their period of feeding on the *Digitaria*. Hoppers which had fed upon a diseased plant of the P.O.J. 213 variety in a similar experiment failed to infect eight *Digitaria* plants.

From diseased *Digitaria* the virus may be transferred to healthy *Digitaria*. Of twelve plants subjected to the feeding of hoppers which had previously fed upon a naturally streaked *Digitaria* plant, one had just developed streak disease, before they were all destroyed in a hailstorm.

The evidence up to this stage suggests no complications in the streak disease relation of *D. horizontalis*. The virus of maize is certainly transmissible to this grass; the virus of cane probably not, although our evidence is insufficient to prove this impossibility. When, however, we studied the transmission from plants of *Digitaria* naturally infected with streak disease, we obtained anomalous results. Hoppers, fed on streaked *Digitaria* plants collected in a site near diseased maize and cane, infected all of nine maize seedlings in an experiment: the pattern of streak was very variable as between different individual seedlings, the spots being of medium to narrow width and the frequency varying from sparse to normal. The virus in this example was certainly less virulent to maize

than the normal maize virus. Hoppers from the same group, when fed upon healthy Uba cane, produced in three out of twelve plants many exceptionally large streaks, but gradually the streaking became more sparse and eventually the plants produced only healthy new leaves. The streaks were of the kind which we obtained in our attempts to transfer the maize virus to cane, but the number of streaks was much greater than we ever obtained in those attempts, and the duration of the diseased phase was much longer. This *Digitaria* virus therefore appeared to be more virulent to cane than the maize virus.

In another experiment, using as the source of infection diseased *Digitaria* plants collected in a maize field, nine out of thirty maize seedlings were infected, all showing the normal full signs except one which was very sparse.

Although, in the preceding experiment, the virus from *Digitaria* had shown a virulence for cane greater than we had ever observed in the maize virus, the following experiment failed to demonstrate any exaltation of virulence by passage through *Digitaria*. Groups of six hoppers each, which had fed upon a *Digitaria* plant experimentally infected from maize, were placed on six healthy cane plants. All cane plants remained healthy except one, which produced a few spots, but not more than might be expected from inoculation by the maize virus direct from maize.

The disease in Eleusine indica *Gaert.*

In the frequency of its infection by streak disease in the field, this species comes next to *D. horizontalis*. We have found diseased plants in many localities, both near diseased cane and particularly in old maize fields. Of particular interest is its occurrence in a maize field in the Northern Transvaal, where no diseased sugar cane was known to grow. The signs of streak in this species are illustrated in Plate XLII, fig. 11, and Plate XLIII, fig. 15.

Although *Eleusine* is so frequently to be seen diseased in maize fields, we have failed to secure clear evidence of the transmission of streak from maize to *Eleusine*. Plants exposed each month in a plot of streaked maize all remained healthy. During this period, however, one self-sown *Eleusine* plant developed streak in this plot.

Attempts to transmit the maize virus experimentally to *Eleusine* all failed. In the eight experiments detailed in Table V, fifty-eight plants all resisted infection, and at no time were even single streaks noted in the experimental plants. We show below, however, that transitory infections

of *Eleusine* may result from transmission from maize which has itself been infected from *Eleusine*.

Table V.

The maize virus—Transmission to Eleusine indica.

Hoppers, fed on diseased maize, caged for 7 days or more on healthy *Eleusine* seedlings, either by glass tubes on single leaves or in lamp-glasses on whole plant.

Date	No. of hoppers to each plant	Method of experiment	Period of observation (days)	No. of plants tested	No. of plants infected	Control plants (healthy)
30. v. 24*	3–4 adults	On single leaf	45	4	Nil	4
13. v. 25*	2–3 adults	,,	60	4	Nil	4
8. vi. 26	5 immature	,,	100	8	Nil	7
19. vii. 26	6 ,,	On whole plant	50	6	Nil	6
20. viii. 26	8 ,,	,,	60	8	Nil	8
28. vii. 27*	4–5 adults and immature	,,	131	12	Nil	12
4. x. 27†	6 adults	,,	63	4	Nil	4
21. v. 28*	2 ,,	On single leaf	88	12	Nil	12

* Hoppers proved infective to maize.
† Hoppers previously fed on *D. horizontalis*, infected from maize.

In a similar way, we failed to transmit the Uba cane virus to *Eleusine*. In three experiments, as shown in Table VI, fifteen *Eleusine* plants resisted infection. On the other hand, one infection resulted in an experiment in transmission from streaked P.O.J. 213. Here the hoppers, after feeding upon the diseased cane, were caged in groups of three upon eight *Eleusine* plants. The one plant showed a rather sparse streaking, as recorded in Plate XLI, fig. 7; but, although a tendency to recovery was later observed, one shoot of this plant was still streaked 7 months after the start of the experiment. A repetition of this experiment afforded no further infections.

Table VI.

The cane virus—Transmission to Eleusine indica.

Hoppers, fed on diseased Uba cane, caged in lamp-glasses upon *Eleusine* seedlings.

Date	No. of hoppers to each plant	Period of observation (days)	No. of plants tested	No. of plants infected	Control plants (healthy)
19. vii. 26	6	50	6	Nil	4
2. xi. 2	6	60	4	Nil	4
16. xi. 27	5	60	5	Nil	5

With the one exception, therefore, our attempts to transmit the maize and cane viruses to *Eleusine* were all failures. It might well be concluded that there is a specialised *Eleusine* streak virus. But if so, we

should have encountered no difficulty in transmission from naturally streaked plants of this species. On the contrary, however, we have not obtained any permanent infections with virus from this source. As shown in Table VII, ten out of thirty-two experimental plants became temporarily diseased, but later recovered. Thus one plant, in the experiment of January 13th, 1928, had streaks on all young leaves 21 days after the start; after 41 days the young leaves were either sparsely streaked or free from streaks; while after 80 days all the young leaves were entirely free from streaks. It is difficult to understand what is the unknown factor which has prevented our infecting a species that is so commonly diseased in the field.

Table VII.

The Eleusine *virus—Transmission to* Eleusine indica.

Hoppers, after feeding upon diseased plants, caged for 7–20 days on healthy *Eleusine* seedlings.

Date	Source of infection	No. of hoppers on each plant	Period of observation (days)	No. of plants tested	No. of plants permanently infected	No. of plants temporarily infected	Control plants (healthy)
4. i. 28	Maize plant, infected from naturally diseased *Eleusine* plant	6	70	12	Nil	8	15
13. i. 28	*Eleusine* plant found naturally diseased in field	3	80	7	Nil	2	9
28. iv. 28	,,	1	88	13	Nil	Nil	13

It remained for us to study the transmission from naturally diseased *Eleusine* to maize and cane. As with *D. horizontalis* we found the *Eleusine* virus to be rather less virulent to maize and more virulent to cane than the maize virus. In one experiment, seven maize seedlings were infected out of eight; none of these plants showed typical full maize streak signs, the disease varying from a medium sparse to a very sparse type. In two later experiments, however, all of sixteen maize plants showed signs indistinguishable from those of normal maize streak. Transmission from naturally streaked *Eleusine* to Uba cane resulted in four temporary infections out of eleven plants tested. These four plants produced from one or two to many streaks similar to those caused in cane by the maize virus. All the plants eventually threw off the disease, although in one the recovery took place only slowly. This plant, 2 months after the feeding of the hoppers, appeared to be normally fully streaked, although the streaks were exceptionally large. After 5 months the streaking had become sparse on the youngest leaves, and the progressive loss of streaking continued until, at the end of 10 months, the plant appeared to be healthy.

At this time the shoots were cut back and the stool allowed to ratoon. The new shoots bore occasional streaks for the first few months, but their frequency diminished until, at 19 months, all young leaves were free from any streak signs. On being again cut back the stool produced only healthy shoots. Three months later these shoots were cut off and planted, but these setts and the original stool produced only healthy shoots. Although therefore the virus appeared eventually to die out, it continued to exert its effect on this plant for more than a year. On no occasion was the maize virus ever observed to maintain such a long protracted infection in Uba cane.

Summary of the experimental results.

The results of the foregoing series of experiments are grouped in Table VIII, in which we indicate not only the measure of success which has followed from attempts at cross-transmission but also the kind of signs produced. The terms used are necessarily somewhat loose, but their meaning will be understood from a study of the photographs accompanying this paper. In considering the width of the streaks we note them as "broad" or "narrow" by comparison with the breadth which we have been accustomed to see in naturally or experimentally infected plants of the species under consideration. Thus Plate XL, fig. 2, shows "broad" and "narrow" streaks in maize; Plate XLI, fig. 7, and Plate XLII, fig. 11, "narrow" and "broad" streaks in *E. indica*. In a similar way the meaning of "high" and "sparse" frequency will be understood from a comparison of the two leaves depicted in Plate XL, fig. 2.

The overwintering of the streak virus of maize in Natal.

The foregoing results suggest that it is not to the perennial host-plants that we are to look for the carrying of the streak virus over from one summer crop of maize to the next. At first sight it was to be expected that sugar cane in particular would act as a reservoir of the virus whence each season's maize would become infected. We have shown, however, that it is highly improbable that the virus of cane may be transferred to maize with the production of the normal disease in the latter. The results of a field experiment support us in this conclusion, for maize planted in the early spring alongside a plot of streaked cane developed at first only the sparse form of the disease. Subsequently a small proportion of the plants became fully streaked; but there is reason to regard this as due to a re-infection by the maize virus, brought about in the following manner. During the previous autumn a maize plot, growing alongside, had been

Table VIII.

Streak virus transferred. Resulting infection.

From Plants naturally infected in the field of the species	To Healthy plants of the species	Positive or negative	Width of streaks	Frequency of streaks	Permanence of infection
Maize	Maize	Positive	Broad	High	Permanent
	Sugar cane, var. Uba	,,	,,	Occasional streaks only	Transitory, no later recurrence
	Sugar cane, var. P.O.J. 213	Negative	—	—	—
	D. horizontalis	Positive	Broad	High	Permanent
	E. indica	Negative	—	—	—
Sugar cane, var. Uba	Maize	Positive	Narrow	Sparse	Permanent, but becoming more sparse with growth
	Sugar cane, var. Uba	,,	,,	Medium	Permanent
	Sugar cane, var. P.O.J. 213	Positive (rarely)	,,	Medium to sparse	Apparently permanent
	D. horizontalis	Negative	—	—	—
	E. indica	,,	—	—	—
Sugar cane, var. P.O.J. 213	Maize	Positive	Narrow	Sparse	Permanent, as from Uba
	Sugar cane, var. Uba	,,	,,	Medium	Permanent
	Sugar cane, var. P.O.J. 213	Positive (rarely)	,,	Medium to sparse	Apparently permanent
	E. indica	Positive (rarely)	,,	Sparse	,, ,,
Sugar cane, var. M.P. 55	Maize	Positive	,,	,,	Permanent, as from Uba
D. horizontalis	Maize	,,	Broad, sometimes narrow	High, sometimes sparse	Permanent
	Sugar cane, var. Uba	,,	Broad	Occasional streaks only	Transitory, but recovery sometimes delayed
	D. horizontalis	,,	,,	High	Permanent
E. indica	Maize	,,	Broad, sometimes narrow	High, sometimes sparse	,,
	Sugar cane, var. Uba	,,	Broad	Occasional streaks only	Transitory, but recovery sometimes delayed
	E. indica	,,	Narrow	Sparse	Transitory, no recurrence

severely streak diseased. Collections of hoppers made during the winter in the cane plot usually contained a small proportion which were carrying a virus fully virulent to maize. We suppose that these individuals were the hoppers which migrated to the cane from the maize, as it died off, and it is to the few survivors that we attribute the infection of the nearby maize in the spring.

We have experimental evidence that the streak virus may overwinter in surviving leafhoppers of the species *Cicadulina mbila*. In May 1925 hoppers, bred on streaked maize, were confined in wire-gauze tubes upon leaves of sugar cane plants in the open at Durban. As necessary the hoppers were moved to fresh leaves, but throughout the winter they remained exposed in the gauze tubes in the open. In October the surviving hoppers were tested on maize. The single survivor of twelve hoppers, caged on healthy cane, produced normal full streak in maize. Of three survivors out of twelve confined on streaked cane, two produced full type streak in maize. Meanwhile, as was to be expected, an originally uninfective hopper, which survived upon streaked cane, caused only sparse streak in maize.

We believe, therefore, that, under the conditions prevailing in the neighbourhood of Durban, the overwintering of the maize virus is due to the insect vector rather than to plant hosts. Probably few infective hoppers actually survive the winter, since early in the season maize may be grown usually without serious losses from streak disease, even in regions where later in the season every plant may be expected rapidly to contract the disease.

Probably also the maize streak virus is carried over in occasional maize plants which survive over the winter in favourable situations. Normally in Natal few maize plants do so survive. But we have demonstrated experimentally the importance of such overwintering maize plants. Through $3\frac{1}{2}$ years in a plot in Durban maize was sown every fortnight. No planting at any season escaped severe streak infection. Unlike plantings in the field in this region, the early spring sowings were no less freely infected than those of the late summer.

Discussion.

The results of our experimental studies show conclusively, we believe, that the virus of streak disease of maize, as it usually occurs in that plant in the field, is not identical with the virus of streak of Uba cane. Each virus shows a specialisation to its own host. The maize virus, producing

a very severe disease in maize and permanent during the life of the plant, is so weakly virulent to sugar cane as to be capable of only a transitory infection of this host. The Uba virus, while readily infecting Uba, but producing in it a comparatively mild though permanent disease, is only weakly virulent to maize. In this host it induces a mild disease, which tends to become suppressed with the growth of the plant.

In thus postulating a difference between the two viruses, we have judged by their effects between host-plants: by the breadth of the chlorotic areas and by their frequency of distribution upon the leaf. We have no doubt that the particular signs produced are characteristic of each virus. In our experiments each virus has produced the expected signs with almost uniform regularity even after repeated passage through alternate host-plants. Variations in the severity of the effects of a virus disease, as between different individual plants, are commonly encountered in virus studies, and are usually attributed to constitutional differences in the plants. We have observed slight variations, which may be due to this cause, but they never approached the magnitude of the differences which we have found between the signs produced in maize by the maize and the cane viruses. It may be noted that with many virus diseases the "severity" of the disease is in a measure to be judged by the contrast between the chlorotic and normal areas in the leaf, that is, by the degree of chlorosis in the affected areas. In streak disease the chlorosis is, except in rare instances, almost complete and the chlorotic areas are almost devoid of chlorophyll. Consequently the total chlorotic area is a rough measure of the "severity" of a streak infection. It has been obvious that there was a relation between the total chlorotic area and the general ill-effects (stunting, etc.) upon the plant.

We believe that our evidence for the recovery of cane plants from a streak infection is trustworthy. Many examples of apparent recovery from a virus infection are explicable merely as masking of signs due to a change of environmental conditions. In such plants the virus is still present. We have never been able to discover any evidence of such masking under the highly variable conditions of our experiments, carried out in the open and in an unheated greenhouse throughout every season of the year. Conceivably, a streak virus may, under certain circumstances, lie dormant in some part of the cane plant. We can only test this possibility by growing the plants under observation for a long period and by testing leafhoppers which have fed upon them. Both these tests have failed with the cane plants which we believe to have recovered from streak infections. We realise, however, that this evidence is not complete. If

a means of artificially transferring the streak virus be discovered, evidence may be forthcoming to cause us to change our view. Within the limitations of the technique available, however, our experiments have shown all the examples of recovery to be absolute.

Our experiments upon wild grasses have been less conclusive. The maize virus may be transmitted to *D. horizontalis* and back to maize without any change of virulence. But in the field *D. horizontalis* may carry a virus which is less virulent to maize than the normal, and a virus which is more virulent to cane than the maize virus, although less virulent to cane than the cane virus. This grass species appears to be susceptible, therefore, to at least one virus which is not identical with either the maize or the cane virus.

The position in *E. indica* is more obscure. It is difficult to understand our failure permanently to infect this species with the virus from naturally diseased *Eleusine*. We know, however, that in the field *E. indica* may be carrying a virus which is fully virulent to maize, or one which is mildly virulent, and a virus which is considerably more virulent to Uba cane than the maize virus.

The trend of our work has been therefore to show that there is not one streak virus but several. We may regard them as different kinds of virus or as strains of one virus specialised to particular hosts. The conception of strains would assume importance only if there were evidence of a change of one strain into another. At present we have no experimental evidence for such a change. At one time we thought we were witnessing the process of adaptation of the streak virus to P.O.J. 213 in the field. But our experiments failed to demonstrate an appreciable change in the virus which had naturally established itself in this cane variety; for the virus from these naturally diseased P.O.J. plants was not notably more virulent to P.O.J. 213 than was the Uba virus. The balance between virus and plant in P.O.J. 213 is, however, at present highly unstable, and at times the plant appears to gain a mastery over the virus. It is not impossible that the virus, now maintaining only a precarious hold upon the plant, may in course of time become specialised to P.O.J. 213 and fully virulent to it.

Definite evidence is, therefore, opposed to the idea that one streak virus may be changed into another. Nevertheless, it can hardly be disputed that the streak viruses form a natural group, clearly differentiated from, for example, the mosaic viruses which affect a nearly similar range of host-plants. For in the first place the signs of the disease in all hosts are similar: discrete chlorotic areas, situated along the veins, unlike the

diffuse mottling of a mosaic disease. Transmission in all the hosts studied is by *Cicadulina mbila*. The disease is essentially an African one (although it has been reported in sugar cane in India and Burma (3, 7)); where the disease occurs in one host it may usually be found in all or most of the other hosts which we have studied. Furthermore, we have to face the fact that the present extensive plantings of Natal Uba originated some 50 years ago almost certainly from a single sett, which was unquestionably healthy. Yet in the intervening years there has appeared a streak virus specialised to Uba cane. On the other hand, in Uganda the recently introduced Uba cane has remained streak-free (4), although, to our own knowledge, streak is there prevalent in maize and *C. mbila* is plentiful. It is possible that there may have existed in Natal, before the Uba cane was introduced, a virus in some unknown wild grass, which found an immediate favourable host in Uba cane. If so, it is surprising that the same virus did not exist in Uganda, and did not at once attack the Uba cane upon its introduction. It is difficult not to believe that in the course of years there has evolved in Natal, perhaps from the maize virus, a strain of streak virus strongly virulent only to Uba cane.

GENERAL SUMMARY.

1. This paper describes experiments in the transmission of streak disease between maize, sugar cane, *D. horizontalis* Willd. and *E. indica* Gaert. The leafhopper, *Cicadulina* (*Balclutha*) *mbila* Naude, acted as the transmitting agent and was usually manipulated by the single-leaf cage method.

2. The virus of maize streak disease is incapable of causing permanent infections of sugar cane. This conclusion is based on a considerable series of leaf-cage experiments, in the greenhouse and in the field, and upon a large-cage experiment. It is confirmed by certain field observations.

3. The maize virus frequently caused transitory infections of Uba cane, in the form of a few large chlorotic streaks on the leaves. From these streaks the virus was re-transferred to maize. All evidence indicated that the cane plant made a complete recovery from this transitory infection and ceased to harbour the virus.

4. The virus from Uba cane readily infected Uba cane. When transferred to maize it produced in this species only a mild form of streak disease, distinguishable with certainty from normal maize streak. Repeated passage of the cane virus through maize failed to enhance its

virulence to maize. Infection by the cane virus afforded no protection to a plant from infection by the maize virus; nor were hoppers similarly protected.

5. Streak disease was discovered in the cane variety P.O.J. 213, hitherto regarded as immune. The virus from these plants produced normal streak in Uba cane and the mild form of streak in maize. After many failures the disease was transmitted experimentally to this variety from Uba cane and from P.O.J. 213 naturally infected in the field. Cuttings taken from diseased plants of P.O.J. 213 frequently produced entirely healthy plants.

6. Lists are given of cane varieties which have been proved to be susceptible to streak disease and of those provisionally regarded as immune.

7. A list is given of species of wild grasses believed to contract streak disease in the field.

8. Streak in *D. horizontalis* has been transmitted from *D. horizontalis* and to and from maize, but not from cane. The virus from a naturally diseased *Digitaria* plant, however, showed a virulence to cane greater than the maize virus.

9. *E. indica* was not successfully infected from maize or Uba cane. It was once mildly infected from P.O.J. 213, and was temporarily infected from *Eleusine*.

10. In experiments infective leafhoppers survived in the open through the winter at Durban and infected maize in the following spring. The infection of maize in the spring in Natal is thought to be caused usually by survival of the virus in overwintering leafhoppers and not in perennial host-plants.

Appendix.

Unsuccessful tests of species of leafhoppers for transmission of streak disease.

Various leafhoppers collected in fields of streak-diseased maize and sugar cane were tested for transmission of streak to maize and cane. These trials, which included twenty-one species of Cicadellids and four species of Fulgorids[1], as detailed in Table XI, resulted in no streak infection. We have no indication of any vector of this disease other than *Cicadulina mbila*.

[1] We are indebted to Dr T. J. Naude for the determination of these insects. The lettering of the unnamed species corresponds to specimens in his collection.

Table IX.

Tests of various leafhoppers for transmission of streak disease.

Combined results of experiments from May 1924–January 1926. No infection by streak disease resulted in any experimental plant.

Name of insect	Collected in diseased cane field		Collected in diseased maize field	
	No. tested on maize	No. tested on Uba cane	No. tested on maize	No. tested on Uba cane
CICADELLIDAE				
Agallia nigrasterna Cogan	3	—	3	—
Balclutha sp. (*b*)	—	17	1	—
Cicadella cosmopolita Signoret	—	—	4	—
,, *spectra* Distant	—	1	—	—
Empoasca sp. (*b*)	—	1	—	—
,, sp. (*c*)	3	—	—	—
Erythroneura sp. (*a*)	19	19	1	—
,, sp. (*b*)	2	1	—	—
,, sp. (*c*)	1	—	—	—
,, sp. (*d*)	1	—	—	—
Eugnathodus auranticulus Naude M.S.S.	20	8	20	—
,, *flavidus* Naude M.S.S.	2	—	—	—
,, sp. (*a*)	2	—	—	—
,, sp. (*b*)	1	—	—	—
Euscelis aethiopica Cogan	—	—	1	—
,, *obscurinervis* Stal.	—	2	—	—
,, *unimaculata* Naude M.S.S.	—	—	3	—
,, sp. (*a*)	—	4	—	—
sp. (*b*)	—	—	1	—
Thamnolettix sp. (*a*)	1	—	—	—
,, sp. (*b*)	3	—	—	—
FULGORIDAE				
Dicranotropis sp. (*b*)	5	1	8	—
,, sp. (*d*)	—	—	1	—
,, sp. (*e*)	1	—	—	—
Perkinsiella saccharicida ,, Kirkaldy	1	2	—	—

In view of the successful results of Stahl (8) in transmitting with *Peregrinus maidis*, a disease of maize in Cuba hardly distinguishable by its signs from our streak disease, we have considered it advisable to test further the possibility that this species may be a vector of African streak disease. Some negative evidence upon this matter has been already presented by one of us (11). In a new experiment one of the large cages already described was filled with forty young maize seedlings. A streak-diseased maize plant, bearing large numbers of individuals of this Fulgorid in all stages of development, was then introduced. At the end of 78 days no plant had developed streak disease. Upon repeating this experiment, 206 plants were healthy at the end of 120 days.

Since it appeared to be possible that the insect used in our experiments might be a different species from the *Peregrinus maidis* of Cuba, we asked Dr C. F. Stahl to supply us with specimens. These were submitted, together with our African specimens, through Mr W. E. China to Dr F. Muir, who found all morphologically identical. Although it is conceivable that there may exist in Cuba a race of *Peregrinus maidis* specialised to the transmission of a virus identical with our streak virus, as suggested by McKinney (6), yet the evidence rather suggests that our streak and Stahl's "yellow stripe disease of corn" are separate diseases. This view is held by Brandes (1) and presumably by Stahl (8), who makes no reference to streak disease in his paper (*loc. cit.*).

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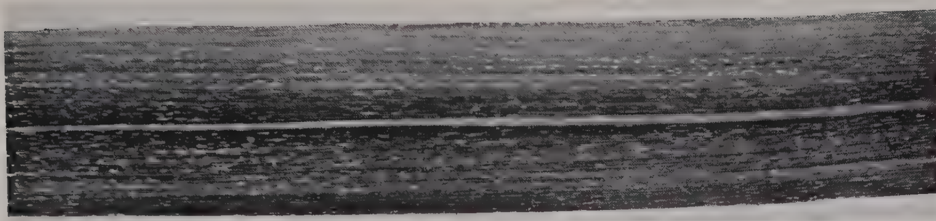

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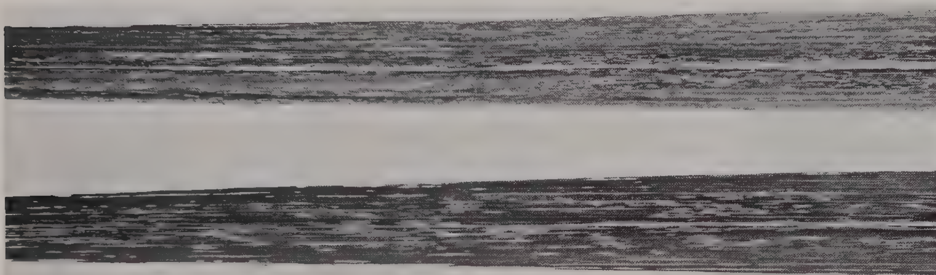

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Fig. 4

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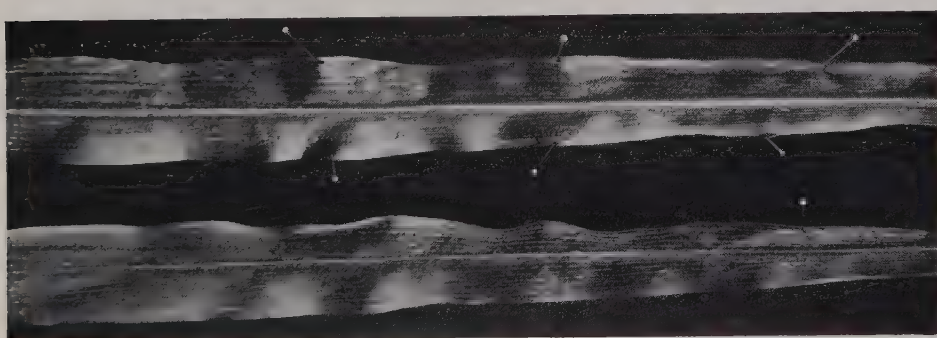

Fig. 3.

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Fig. 2.

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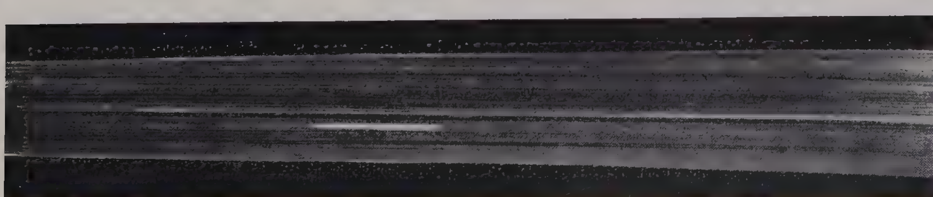

Fig. 1.

STOREY & McCLEAN.—The Transmission of Streak Disease between Maize, etc. (pp. 691–719).

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Fig. 6.

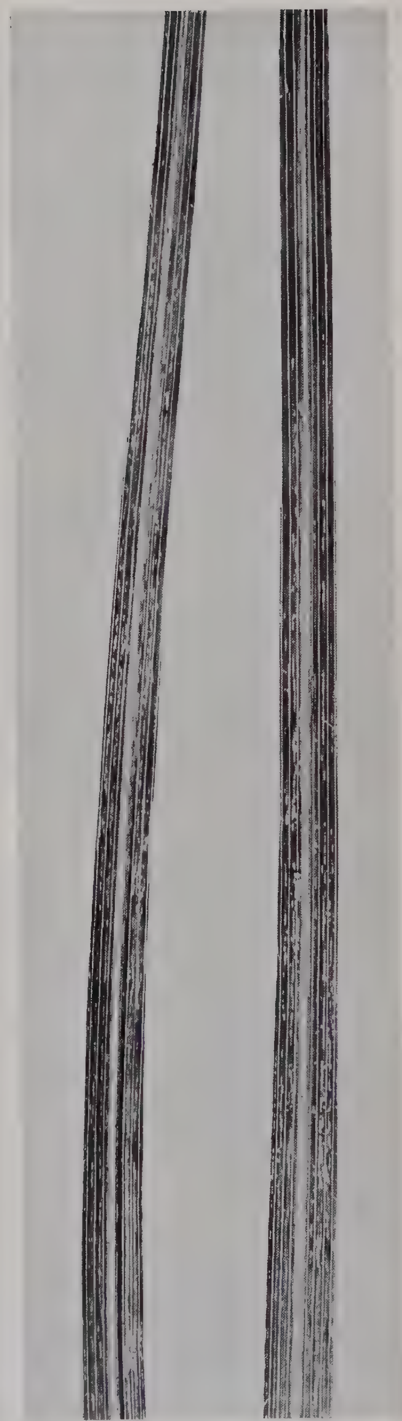

Fig. 7.

STOREY & McCLEAN.—The Transmission of Streak Disease between Maize, etc. (pp. 691–719).

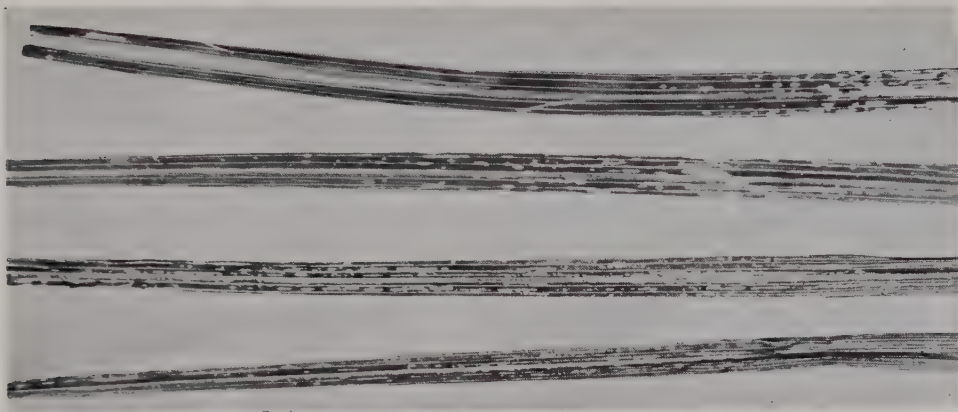

Fig. 11.

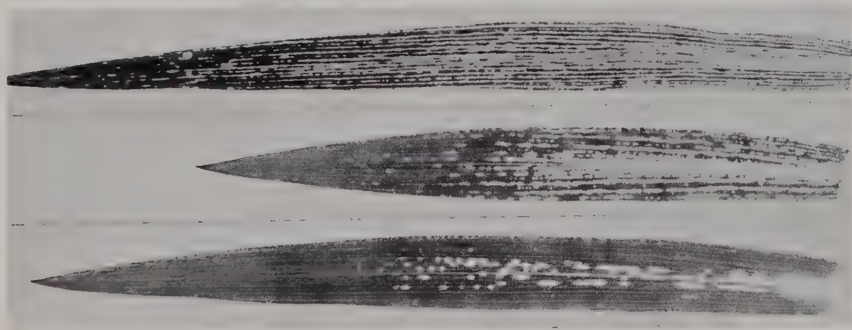

Fig. 10.

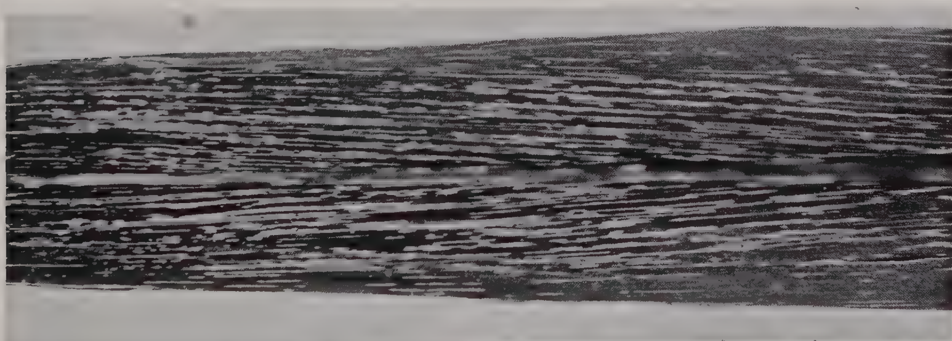

Fig. 9.

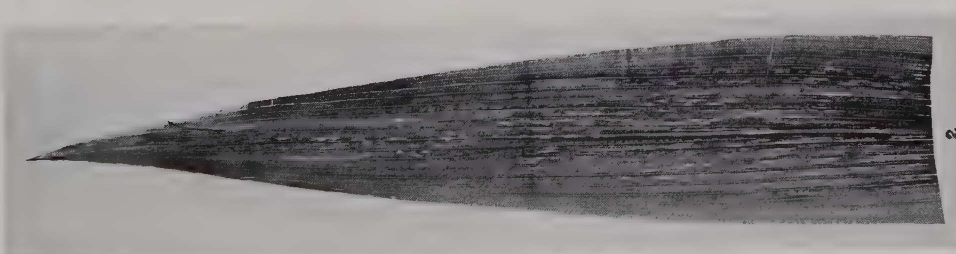

Fig. 8.

STOREY & McCLEAN.—The Transmission of Streak Disease between Maize, etc. (pp. 691–719).

Fig. 12.

Fig. 13.

Fig. 14.

Fig. 15.

STOREY & McCLEAN.—The Transmission of Streak Disease between Maize, etc. (pp. 691–719).

EXPLANATION OF PLATES XL—XLIII

PLATE XL.

Fig. 1. Leaf of Uba cane plant experimentally infected with the virus from streaked maize, showing single large chlorotic streak. × $1\frac{1}{2}$.

Fig. 2. Leaves of maize plants, experimentally infected (1) with streak virus from maize, (2) with streak virus from Uba cane. × $\frac{1}{2}$.

Fig. 3. Leaves of maize plant, experimentally infected by the virus, originally obtained from Uba cane, but passed three times through maize. × $\frac{1}{2}$.

Fig. 4. Leaves of sugar cane plants naturally infected with streak disease: (1) P.O.J. 213 variety, showing about the maximum frequency of streaking observed, and (2) Uba variety, normal frequency. × $\frac{2}{3}$.

Fig. 5. Leaf of Uba cane plant, experimentally infected from P.O.J. 213, showing normal frequency of streaking. × $\frac{2}{3}$.

PLATE XLI.

Fig. 6. Successive alternate leaves taken from a maize plant, originally experimentally infected from Uba cane and later infected from maize. These leaves show (1) the sparse streaking of the Uba virus, (2) the same, but with the normal full streaking towards the base of the leaf, and (3) normal full streaking. × $\frac{1}{2}$.

Fig. 7. Leaves from a plant of *E. indica*, experimentally infected from streaked P.O.J. 213 cane. × $1\frac{1}{3}$.

PLATE XLII.

Fig. 8. Leaf of maize plant, experimentally infected from streaked P.O.J. 213 cane. × $\frac{2}{3}$.

Fig. 9. Leaf of sugar cane, variety Black Tanna, naturally infected with streak disease. × $\frac{1}{2}$.

Fig. 10. Leaves of *D. horizontalis*, naturally infected with streak disease. × $1\frac{1}{4}$.

Fig. 11. Leaves of *E. indica*, naturally infected with streak disease. × 1.

PLATE XLIII.

Fig. 12. Enlarged photograph, by transmitted light, of streaks on a maize leaf, infected with the maize virus.

Fig. 13. Enlarged photograph, by transmitted light, of streaks on a maize leaf, infected with the Uba virus.

Fig. 14. Plant of *D. horizontalis*, naturally infected with streak disease.

Fig. 15. Plant of *E. indica*, naturally severely infected with streak disease.

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A NEW STRAIN OF *TILLETIA TRITICI* IN PALESTINE

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In a former publication (7) on comparative bunt resistance of wheat in Palestine, the results of two years' inoculations of twenty-two local and foreign wheat varieties with *Tilletia tritici* were given. All the varieties tested proved highly susceptible to *T. tritici* with the exception of two Australian wheats, Florence and Bunyip. The former, which is known to be slightly susceptible to *T. tritici* in Australia (4, 5) and in the United States (15, 16), showed absolute immunity; Bunyip, which in the same countries (8, 16) is highly susceptible, in one year's experiments in Palestine was immune and in another year highly resistant, though the other varieties tested showed up to 70 per cent. infection. These results were both striking and important from a practical point of view, since both wheats could safely be introduced to local agriculture or used for breeding.

The question then arose whether the immunity or resistance shown was temporary or permanent. The results of Sessous' (13), Roemer's (11) and Gaines' (2) experiments showed that the American immune varieties Martin, Hussar and Ridit were highly susceptible to bunt of German origin. Furthermore, Faris (1), Rodenhiser and Stakman (10), and Reed (6) have shown that *T. tritici* and *T. leavis* comprise distinct physiologic forms in different countries which possess a different degree of virulence towards certain wheat varieties. Great care had therefore to be exercised, in the light of the results obtained, before drawing conclusions as to the permanence of this resistance.

The immunity and resistance of these two varieties could thus be accounted for in two ways: (1) the local Palestine strain of *T. tritici*, unlike the American and Australian strains, might be incapable of attacking these varieties or, (2) the two wheat varieties themselves might prove to be resistant strains of susceptible varieties. The existence of such resistant strains in susceptible wheat varieties, especially when the former derive from different stocks, can already be found in the classical bunt-resistance experiments of Kirchner, which the author himself overlooked (3, 7); further, this fact has been recently experimentally proved

by Stadler (14). A special investigation was therefore undertaken in 1928–9 to ascertain the real causes of the different behaviour of Florence and Bunyip in Palestine.

MATERIALS AND METHODS.

In order to carry out this experiment different sources of *T. tritici* material and another stock of the Florence and Bunyip varieties were needed. As it was desired to conduct the experiment during 1929 there was no time to get material from the U.S.A. or Australia, but Miss Kathleen Sampson, Aberystwyth, Wales, and Dr Theodor Roemer, Halle, Germany, who used Florence wheat and different collections of *T. tritici* in their experiments, were so kind as to supply me with the necessary material. Miss Sampson sent Florence seeds and *T. tritici* spores used by her, while Dr Roemer furnished me with seven different collections of *T. tritici*: one collection from the U.S.A., one from Holland, one from Sweden, one from Denmark, one from Switzerland and two from Germany. Including the local and Welsh collections we had, altogether, nine collections of *T. tritici* ready for use.

The following wheat varieties were used: Florence variety grown in Palestine and called Florence Palestine, Florence variety sent by Miss Sampson called Florence Wales, and Bunyip grown in Palestine. We were unable to obtain another stock of the latter variety.

The seeds were contaminated in a container with bunt spores taken from infected wild emmer plants and were sown in rows of fifty seeds on December 27th, 1928. They were sown 15 cm. apart and spaced 7·5 cm. apart. The soil in which the seeds were sown was sufficiently moist, the rainfall for the last ten days before sowing amounting to 33 mm. Rain fell again three days after sowing, and the total amount during the next eight days reached about 80 mm. The temperature was thus reduced to 10° C. at the time of germination.

Many of the plants were unfortunately damaged by field mice, but the remaining plants sufficed to give a clear idea of the results.

From the following experimental results we see that the absolute immunity of the Florence Palestine wheat was broken by three collections of bunt, namely, Breslau, Wageningen and Zurich. The Florence Wales wheat variety was infected only by the Breslau collection. The Breslau bunt collection was thus the most virulent, infecting the Florence Palestine variety up to 7·7 per cent. and the Florence Wales variety up to 9·1 per cent. The bunt collection of Zurich infected only the Florence

Palestine variety up to 3·8 per cent. The Wageningen collection was the least virulent, infecting only the Palestine variety up to 2·4 per cent.

Table I.

The percentage of smutted plants obtained in two stocks of Florence and one of Bunyip inoculated with nine collections of Tilletia tritici. *Experiment conducted at the Experiment Farm, Gevath, Palestine,* 1928–9.

No.	Source of inoculum	Florence Palestine			Florence Wales			Bunyip		
		No. plants	No. infected	% infected	No. plants	No. infected	% infected	No. plants	No. infected	% infected
1	Aberystwyth, Wales	22	0	0	14	0	0	12	5	41·6
2	Pullman, U.S.A.	7	0	0	—	—	—	15	2	13·3
3	Cosel, Germany	—	—	—	—	—	—	7	1	14·3
4	Breslau, Germany	39	3	7·7	11	1	9·1	29	13	44·8
5	Wageningen, Holland	42	1	2·4	12	0	0	34	2	5·9
6	Landeskrone, Sweden	32	0	0	9	0	0	28	3	10·7
7	Lyngby, Denmark	19	0	0	4	0	0	3	1	33·3
8	Zurich, Switzerland	26	1	3·8	10	0	0	21	0	0
9	Palestine	14	0	0	7	0	0	10	0	0

The resistance of the Bunyip wheat variety was broken by nearly all the foreign collections used, with the exception of the Zurich collection. The bunt strain most virulent towards Bunyip was, as in the case of Florence, the Breslau collection, which caused an infection of 44·8 per cent. The next most virulent collection was that from Wales, producing an infection of 41·6 per cent. The third strongest collection on Bunyip was that of Lyngby (Denmark), which caused 33·3 per cent. infection. The Cosel (Germany), Pullman (U.S.A.) and Landeskrone (Sweden) collections induced a smaller, almost equal degree of infection (14·3, 13·3 and 10·7 per cent. respectively). The lowest degree of virulence on Bunyip was shown by Wageningen, which induced only 5·9 per cent. infection.

The Palestine bunt collection did not, as in former years, infect either Florence Palestine or Florence Wales. Even Bunyip, which in one of the former years was slightly infected, remained immune this year. The viability of the Palestine collection was proved by its capacity for infecting local wheat varieties sown on the next day.

The virulence of the collections in different countries.

It may be of interest to compare the degree of virulence shown by the foreign bunt collections in Palestine with that shown in other countries. Thus, the Breslau collection, which was one of the more virulent in our trials, was also the strongest of all the same six collections in Roemer's experiments. The second most virulent strain in our experiments was that from Zurich, a result differing from those of Roemer's experiments in which the Wageningen collection proved more powerful. In Roemer's experiments also the Pullman collection was the weakest, while the Lyngby and Landeskrone collections, which showed no virulence towards three varieties tested in Palestine, were stronger in Roemer's experiments than the Zurich collection. The Wales collection, which failed to infect either of the Florence stocks used in the Palestine tests, was virulent enough in Miss Sampson's experiments (12) to infect the Welsh Florence stock. The reason for such a change is difficult to explain, but climatic and ecological causes may perhaps be involved.

Conclusions.

A conclusive explanation of the cause of the immunity from, and resistance to bunt of Florence and Bunyip wheat varieties in Palestine was furnished by the results of three years' experiments.

It was shown that their immunity is a local one and limited to the strain of *T. tritici* prevalent in Palestine, and possibly also to the Danish collection. The U.S. and Wales collections were able to infect Florence in the respective countries (12, 16). The German, Dutch and Swiss collections succeeded in breaking the immunity of the Florence and Bunyip varieties in Palestine.

The Palestine collection of *T. tritici* may therefore be considered as a new strain.

On the whole, the different foreign collections of bunt were found to maintain their virulence also in Palestine, where climatic conditions differ from those in the countries of origin.

Ecological and climatic factors may, however, have exerted some influence on the virulence of the foreign collections. Thus, the Welsh collection, which was sufficiently virulent to infect Florence in Wales, could not attack either the local or the Wales stock in Palestine.

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BACTERIAL DISEASES OF STONE-FRUIT TREES IN BRITAIN

II. BACTERIAL SHOOT WILT OF PLUM TREES

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(With Plates XLIV–XLVII.)

Introduction.

The subject of the present communication is a disease of plum trees which came under the author's notice as long ago as 1923. The delay in publishing a detailed account of the results obtained is due to two causes. In the first place, although the organism causing the disease may be isolated without difficulty from infected material, it apparently requires special environmental conditions for infection to be conspicuous, and unless these conditions prevail, inoculation experiments, carried out to test the pathogenicity of the organism, may give inconclusive results. The second cause of delay is owing to the fact that the organism shows a tendency to divert from the type when kept in cultivation. At first this was thought to be a result of imperfect isolation or of contamination, so that the purity of the cultures was in question, but it is now believed to be a case of "saltation" or "dissociation." Such divergence from type in bacterial cultures is by no means rare, as recent papers on the subject have shown (3, 4, 7).

The disease.

A very brief account of bacterial wilt has already appeared (9), but a more detailed account is now desirable. Towards the end of May 1923 the author's attention was drawn to a number of Victoria plum trees growing in nursery rows on the East Malling Research Station. Most of the trees showed a wilting of one or more of the young green shoots. The trees had been well cultivated and treated so as to make vigorous growth. They had been grafted low in 1922 and, during that year, each had produced a stout stem about 6 ft. long. During the winter of 1922–3 these stems had been cut back about a foot, so that in May 1923 each tree had

a stem 5 ft. long and a head of five or six long green leafy shoots; these shoots, that normally would have developed into the main branches of the mature trees, were the ones killed back by this wilt disease. Although the shoots were long and stout they had grown quickly during a period of wet weather and were distinctly sappy. These conditions appear to be those conducive to epidemic infection, and in confirmation it may here be observed that in the inoculation experiments on young shoots, described below, successful inoculations resulting in wilting were obtained only on shoots growing rapidly in a moist atmosphere.

Although bacterial infection spots were present on many of the leaves, the wilting of the shoots was caused by direct infection of the axis of the shoots, and not to infection through the leaves. On some infected shoots the terminal leaves though flaccid and drooping were still green. Many of the shoots bore a number of isolated, blackened, usually slightly sunken, spots, and these were evidently early stages of infection. Small spots (2 to 3 mm. in length) were elliptical, slightly sunken, and each had a dark (almost black) centre, surrounded by a paler zone which was bordered by a darker line (Plate XLV, fig. 4). The spots became elongated lengthwise to the shoots and often extended for several inches along one side (Plate XLV, fig. 3). On some shoots the lesion was wholly on one side, not girdling, and the apex of such a shoot, though not wilted, became recurved in consequence.

A microscopic examination showed the blackened areas to be crowded with rod-shaped bacteria which oozed out in dense masses when a particle of the infected tissues was teased out in water.

The disease bears a superficial resemblance to wither-tip of plum trees caused by the brown-rot fungus *Sclerotinia cinerea* (8). In each there is a discoloured area on the axis of the shoot, and wilting of that part of the shoot distal to the lesion. Bacterial wilt differs from wither-tip, however, in that the lesions typically originate on the axis and do not, so far as observations have gone, invade the shoots from infected leaves as in the brown-rot wilt. The bacterial lesions are more elongated, often several inches long, before girdling the shoots, whereas in wither-tip the lesions girdle while still short, so that the upper and lower limits are almost transverse to the axis. Microscopically the presence of the dense bacterial masses in the lesions is a diagnostic character of the bacterial wilt.

The bacteria have not been observed to ooze out of the lesions as globules or slimy masses in the open, but if shoots are cut off, and kept with their cut ends in water, in an atmosphere saturated with moisture,

drops of a yellowish liquid may appear on the surface of the lesions and these drops swarm with bacteria. Plate XLV, fig. 5, shows a portion of one of these shoots after being kept with its lower end in a beaker of water and covered with a bell-jar for 48 hours. From one of these drops the organism was isolated and later proved to be pathogenic.

After the observations recorded above were taken the infected shoots were removed from the trees, but in the following year the disease again appeared on the same trees, though less severe than in 1923.

This disease, associated with the presence in the lesions of the organism described below, has not been met with on naturally infected plum shoots since 1924, but lesions of a very similar nature, though less conspicuous, have been observed from time to time on plum trees. The organism isolated from these more recently observed lesions differs from that causing the shoot wilt described in the present paper in certain cultural characters and is indistinguishable from the bacterial canker organism which will be described in a later article.

Inoculation experiments.

The inoculation experiments have been carried out chiefly with the strains numbered as follows:

A II, isolated in 1923 from a drop of bacterial ooze obtained from a naturally infected shoot which had been cut off and kept in a moist atmosphere for two days.

A II R, isolated in 1928 from a lesion on a shoot which had been inoculated with A II.

A V, isolated in 1924 from a lesion on a naturally infected shoot.

Numerous inoculations have been made with these strains. Some of these experiments have failed to give positive results, and many have produced obvious lesions which, however, did not extend far and therefore did not cause wilting. In the open it is not often possible to obtain the environmental conditions necessary for the infection to run its course, and under ordinary greenhouse conditions the shoots usually harden up so quickly that infection fails completely. Inoculations on shoots in the greenhouse have been successful only on young trees which were kept in a confined space, with a saturated atmosphere, for several days. The inoculation experiments described below were carried out on trees in the open unless the contrary is stated.

On buds.

26/6[1]. In March, just as the buds were swelling, five buds on one shoot were inoculated by placing a drop of bacterial suspension (in water) on each and puncturing the bud through the drop. On a control shoot five buds were punctured without inoculation. Eighteen days later the punctured control buds had all grown out normally, except that some of the leaves showed mechanical injury due to the puncturing. All the inoculated buds were dead. Inoculations on uninjured buds gave negative results in this experiment.

On leaves.

25/19. Three leaves on each of four shoots of a tree in the greenhouse were inoculated at punctures, six punctures on each leaf; leaves on four other shoots were punctured without inoculation. In 6 days all the inoculated spots showed a narrow black border bounded by a yellow zone, 1 to 2 mm. wide. The control punctures showed a narrow pale border only and no yellowing.

25/20. Six leaves on a tree in the open were inoculated at four punctures each. Six other leaves were punctured but not inoculated. Observations were made 6 weeks later when the control punctures were seen as holes up to 1 mm. in diameter, while the inoculated punctures had given rise to dead patches of tissue which had fallen or were about to fall away leaving "shot holes" from 2 to 10 mm. in diameter.

26/10. Six leaves were inoculated on a tree in the greenhouse. On each of these leaves four drops of bacterial suspension were placed on one side of the midrib and four drops of sterile water on the other side of the midrib; a sterilised needle was then pushed through each drop puncturing the leaf. In 6 days each inoculated spot had a blackened margin, a feature not shown by the control punctures. On the 14th day the infected spots were 3 mm. in diameter and the dead tissues were by this time becoming separated from the healthy (see Plate XLVI, fig. 6).

29/11. The strain used in this experiment was A II R (isolated in 1928 from a shoot inoculated with A II); it had been in culture about a year when used in this experiment. Shoots on three plum trees were sprayed with a bacterial suspension of the organism; the leaves were not punctured or otherwise injured. An equal number of shoots on the same trees were sprayed with sterile water as controls. The results are shown in Table I.

[1] Each experiment is denoted by two numbers, the first indicating the year in which the experiment was carried out, the second being the serial number of the experiment.

Table I.

Tree	No. of shoots inoculated	Result 13 days later
1	1	2 leaves show spotting
2	5	1 or 2 leaves on each of the five shoots show spotting or "shot-holes"
3	2	Each shoot shows five leaves with spots or "shot-holes"

The number of spots or "shot-holes" was one to eleven per leaf. No spotting was to be found on any of the control shoots. The organism was isolated from one of the spots as strain A II RR which in culture was found to behave as A II and A II R. One of the infected leaves is shown in Plate XLVI, fig. 7.

On the fruit.

Inoculations on young green plums on trees in the greenhouse produced sunken discoloured areas, extending round the points of inoculation, within a few days. One of these experiments, 24/3, is illustrated in Plate XLVI, fig. 8. A cluster of three plums was selected. The middle one was punctured at two places but not inoculated; the other two were inoculated at punctures. In 7 days the inoculated spots had each a sunken discoloured zone extending 1 to 2 mm. from the punctures. The control spots showed brown cells only at the actual puncture.

On shoots.

24/1. Three shoots were inoculated at punctures made with the point of a scalpel, and two shoots were punctured without inoculation; each shoot was punctured at two places. Within 9 days the six inoculated spots had developed into blackened lesions from 0·6 to 1·5 cm. long; the control punctures showed no discoloration apart from the brown dead cells bordering each. Further progress of infection was checked except at one inoculated spot where the lesion, 8 days later, was a black irregular streak, 3 cm. long.

25/4. Eight shoots on a young plum tree were each punctured at one spot; on four of the shoots the punctures were inoculated; the other four were not inoculated. At the end of 14 days each inoculated puncture had become a black elongated lesion about 4 mm. long, and the condition of inoculated shoots was as follows: (1) Shoot not seriously affected, lesion drying up. (2) Tip of shoot blackened and dead (Plate XLVI, fig. 9). (3) Distinct distortion of that portion of the shoot distal to the lesion.

(4) Distortion above the lesion. The control shoots showed punctures only; there was no blackening and no distortion.

25/11. Four shoots were inoculated and four were controls, each with one puncture. The inoculated punctures became black lesions 4–10 mm. long, causing distortion of the shoots within 12 days.

25/13. Four shoots inoculated, four controls, as in Exp. 25/11. Inoculated punctures became lesions 5 to 10 mm. long in 9 days, with pronounced distortion in three of them.

25/18. A young plum tree (var. Brompton) in the greenhouse had been cut back so as to induce vigorously growing shoots and the tree was kept in a moist atmosphere. Four shoots developed, two of these were inoculated, and two were controls, each with three punctures. In 2 days all the inoculated punctures had developed into blackened lesions 3 to 5 mm. long and the two shoots were wilting towards the apex. On the 6th day each of the inoculated shoots showed an infected portion about 4 cm. long connecting the punctures and extending upwards and downwards from the punctured region; the apical portion had collapsed while the leaf below the lowest puncture, and situated on the infected area, showed discoloration of the midrib and the main veins (Plate XLVII, fig. 10).

25/21. Of nine shoots on a young plum tree, five were inoculated and four controls, one puncture on each. In 3 days all the inoculated shoots had black lesions 1 to 4 mm. long, the controls showing, at the punctures, a slight brown discoloration only. On the 6th day the tip of each of three inoculated shoots was wilting, in the other two there was a black lesion 1 cm. long. One of the wilting shoots had a lesion extending along one side for 3 cm. downwards from the point of inoculation; this lesion eventually reached a length of 16 cm. (Plate XLVII, fig. 11).

28/11. In this experiment strain A II was used. Five shoots were inoculated and five controls, each with one puncture. The weather was too dry for good infection, but in 15 days three of the inoculated shoots showed black lesions 3 to 10 mm. long with distortion of the shoots. From one of these lesions the organism was re-isolated as strain A II R (used in Exp. 29/11 on leaves).

On twigs and branches.

On woody twigs and branches inoculations were made by inserting a drop of a bacterial suspension (in water) in a small cut in the bark.

25/10. In April 1925 inoculations were made on one-year-old plum twigs. Four weeks later the inoculated spots had developed into sunken

areas extending 2 to 4 mm. above and below the points of inoculation. The lesions later became covered with callus, but not before some of them had developed into small cankers 1 to 2 cm. long and half girdling the twigs. Control wounds did not become sunken or elongated and soon became covered with callus.

26/24. Inoculations made in October 1926: on one tree two branches were each inoculated at two places, and on another tree four branches at one place each. In the following April the inoculated wounds had developed into sunken areas of bark extending 1 cm. upwards and 1 cm. downwards from the point of inoculation. Gum was present on each inoculated spot. The control wounds showed no sunken areas of bark and no visible gum. In July the infected areas were small elongated cankers from 2 to 6 cm. long, but by this time they were covered or nearly covered with callus.

On stems.

Inoculations on stems with the wilt organism have usually produced some disturbance, but in no case has a large canker developed.

26/3. In March five young plum trees were inoculated, three of them at two places, the other two at one place each; five trees were cut without inoculation. In April the bark was slightly sunken round the cuts on the inoculated trees. Later these inoculations developed into wide open lesions, 2 to 3 cm. long, glistening with gum, but by July they were covered with callus. The control wounds did not increase in size and were soon covered with callus.

In another experiment (26/5) young trees in pots were similarly inoculated on the stems. The inoculations produced slight sinking of the bark round the wounds and copious gum. Control wounds became normally covered with callus; there was no sinking of the bark and very little or no gum.

26/26. This experiment was carried out in October 1926 on the trees used for Exp. 26/3, the former wounds being completely healed by this time. The inoculations were made as in 26/3. The inoculated wounds developed into cankers 3 to 8 cm. long, some of them with gum; the control wounds healed normally without gumming.

Discussion on results of inoculations.

In the inoculation experiments recorded above infection arose only at wounds, except in one case where leaf-spotting was induced by spraying the leaves with a bacterial suspension. Even in the open, where

infection had arisen at a puncture on a shoot and there was opportunity for the organism to get washed by rain to other parts of the shoots, there was no clear evidence that infection had arisen anywhere but at the actual inoculated spot. Observations, on naturally infected shoots which have shown a number of lesions on a single shoot, have suggested that infection may arise without previous mechanical injury, but experiments have not yet confirmed this. Whether biting or piercing insects contribute in inducing infection is a point on which observations have not yet been made.

Inoculations on the woody parts of plum trees have usually given rise to an exudation of gum at the inoculated wounds, while control wounds have become healed without any conspicuous gummosis, though occasionally a little gum appears. There is good evidence, therefore, that the bacterial wilt organism is one of the causes of gummosis, although this particular organism has not yet been found associated with naturally infected lesions on woody branches or stems of plum trees.

Inoculations on woody twigs, branches and stems, when carried out in the autumn, have yielded more striking results than when made in the spring, but these particular experiments have not produced cankers severe enough to kill the parts distal to them. It was thought at first that a study of the bacterial shoot wilt would supply some evidence as to the cause of the so-called "die-back" disease of plum trees, especially as bacteria were associated with lesions found on trees showing die-back symptoms, and the fact that this organism can infect woody branches and stems (as shown by the inoculation experiments) lends some support to this. An organism that has been isolated from a number of dying trees differs from the one described here in certain cultural characters, and appears also to be more destructive when inoculated into branches and stems. A detailed account of the organism causing "bacterial canker" of plum trees is reserved for a future communication.

Description of the organism.

The organism which has been isolated from naturally infected shoots, and re-isolated from artificially infected shoots which had been inoculated with pure cultures, has the following characters.

Morphology. Taken from 2-day-old cultures on nutrient agar slopes, fixed with formalin and stained with methyl violet, the organism is seen as rods with rounded ends 0·9 to 2·5 μ long and 0·3 to 0·5 μ wide in rods not showing constrictions. The rods show a tendency to cling together in chains of two or more, sometimes forming filaments; the longest filament

seen on such slides was about $50\,\mu$ long. The organism stains well with methyl violet, carbol fuchsin, gentian violet, aniline gentian violet and rather faintly with methylene blue and bismarck brown.

It is stained, but not deeply, by Gram's stain, following the method given by Eyre[1] and also by the "usual procedure"[2] using aniline gentian violet or carbol gentian violet.

The presence of flagella was demonstrated by Plimmer and Paine's, Gray's, and Moore's methods. There is usually one polar flagellum, about twice the length of the rod, but many rods show two or three. They are generally at one pole but, in the longer rods, they may be present at both poles, such rods probably being in process of dividing.

Staining by Ribbert's method failed to show capsules, but on slides stained by Plimmer and Paine's flagella stain it was seen that, in many individuals, the flagella were attached to a hyaline sheath surrounding the rod and not to the deeply stained body of the rod.

No spores have been observed, and to prove their absence two tests were applied: (*a*) Tubes of nutrient broth were inoculated from cultures of nutrient broth and saccharose (5 per cent.), 5 weeks old, growing at room temperature; half the tubes were heated at 85° C. for 10 minutes, the other half were not heated. All the tubes were then incubated. Growth appeared only in those tubes which had not been heated. (*b*) Bacterial slime was transferred from nutrient agar slopes, 7 days old at 25° C., to nutrient broth. These were heated at 85° C. for 10 minutes after making transfers from them to other tubes as controls to show viability of the original cultures. All the control tubes produced growth, while no growth appeared in the heated tubes.

T.D.P. That the organism is killed by temperatures much lower than that used for testing for spores was shown in determining the thermal death point. Preliminary trials showed that the t.d.p. was in the neighbourhood of 45° C., so tests were carried out at temperatures of 44°, 45° and 46° C. Small test tubes ($\frac{1}{2}$ in. in diameter) containing 3 c.c. of sterile water were inoculated from nutrient agar cultures 8 days old (at 25° C.) so as to make a slightly turbid suspension and heated at the temperatures mentioned for 10 minutes; tubes of nutrient broth were then inoculated from them. No growth appeared in nutrient broth inoculated from the tubes heated at 46° C.; nutrient broth inoculated from the tubes heated at 45° C. produced growth, but it was retarded compared with that in broth inoculated from the tubes heated at 44° C. Under the conditions

[1] *Bacteriological Technique*, 2nd ed. 1913, p. 108.

[2] *Manual of Methods.* Society of American Bacteriologists, Supplement A, p. 7.

of the experiment 46° C. was the t.d.p. though 45° C. evidently had an adverse effect on the organism.

Optimum temperature. This has not been determined with any degree of accuracy, but the organism has been grown simultaneously at (*a*) room temperature, 15°–18° C., (*b*) 20° C., and (*c*) 25° C. The organism has invariably made most rapid growth at 25° C. and developed more vigorously at 20° C. than at room temperature.

Cultural characters.

The organism when growing in mass on agar slopes or plates is greyish or yellowish white, while on sterilised potato plugs the yellowish tinge is more evident. It must, however, be classified with the white organisms, as its yellowness is of a different order from that of the true yellow organisms such as, for example, *Pseudomonas pruni*, which attacks plum trees in America.

It is strongly aërobic, as shown by the non-clouding of the liquid in the closed arm of fermentation tubes. Moreover, in liquid media, growth is at first most noticeable in the upper parts of the liquid, and usually a pellicle appears at the surface within a few days.

The characters noted below are for cultures incubated at 25° C. unless otherwise stated, except gelatin cultures, which were incubated at 20° C.

Nutrient broth (Difco). There is a little cloudiness within 24 hours; in 48 hours the cloudiness is more pronounced and a thin readily fragmenting pellicle is present. Sometimes there is a very fragile pellicle within 24 hours, but it is more evident if the cultures are left undisturbed for 48 hours; on moving the tubes even slightly, the pellicle is then seen to fragment and fall in small flakes.

Beef extract (from fresh meat) with peptone. Growth is very much as in nutrient broth, except that a distinct yellowish tinge appears in beef extract (not shown or only very faintly indicated in nutrient broth), so that the liquid eventually is almost lemon yellow in colour.

In nutrient broth containing dextrose, lactose, saccharose (1 per cent.) or glycerine (2 per cent.) there is good growth, but in Durham's tubes the growth does not extend into the inner, inverted tube, and no gas appears in the latter.

Dextrose litmus broth. Reddening of the liquid appears in 2 or 3 days, indicating acid production; later there is reduction of the litmus and the liquid becomes yellowish.

Lactose litmus broth. This medium shows an alkaline reaction within a few days; later there is reduction of the litmus.

Saccharose litmus broth. There is generally a slight reddening within 2 to 4 days, but later the reaction is masked by the reduction of the litmus. In tests where the tubes have been kept for a month or more the neutral purple colour has returned, the reaction sometimes becoming even more alkaline than control tubes.

Glycerine litmus broth. Reaction slightly alkaline within a few days; later reduction of the litmus causes a yellowing of the liquid.

Indicator broth. These above-mentioned sugars and glycerine were also used in broth cultures containing bromo-cresol purple and cresol red as a combined indicator for change of reaction; for comparison, tubes of nutrient broth containing only the indicator were also inoculated. The nutrient broth alone showed a slight alkaline reaction in 1 to 2 days, and this alkalinity was clearly seen within 4 days. The dextrose broth showed slight acid reaction in 24 hours and acidity was clearly indicated within a few days. Lactose broth became alkaline in a few days, the colour change being about equal to that in nutrient broth without sugar. Saccharose broth became acid within a few days, but later, as in saccharose litmus broth, there was indication that the reaction became reversed. In glycerine broth the reaction was alkaline, but not so clearly shown as in lactose broth.

The reaction in broth with saccharose or glycerine was not clearly marked by the bromo-cresol purple and cresol red indicator, and it was masked by dichromatism, so that the reaction appeared to be an acid one by transmitted light and alkaline by reflected light. To avoid such dichromatism bromo-phenol red[1] was used as indicator. This showed the reaction more clearly, but tended to become very pale in cultures that maintained a reaction in the neighbourhood of pH 7. In the glycerine broth cultures there was definite alkaline reaction in a few days, and this persisted. In the saccharose broth cultures there was, on the whole, a slight acid reaction at first, followed sometimes by a reversal of the reaction.

This change from neutrality to acidity and reversal of the reaction was best shown, however, by making use of the "capillator" method of testing for pH. Tubes of nutrient broth containing 1 per cent. saccharose were inoculated and incubated at 25° C. It was found that samples sufficient for testing by this method could be removed without contamination, by means of a large platinum loop, so that the same cultures could

[1] This indicator is recommended, in the *Manual of Methods* issued by the Society of American Bacteriologists, for replacing bromo-cresol purple when the latter gives trouble from dichromatism. The author was unable to obtain it in this country, but after consultation with Prof. H. J. Conn eventually obtained a sample from America.

be used throughout the experiment. A control tube remained sterile although samples were removed weekly over a period of 15 weeks. It was found that, at the end of the first week, there was a slight acid reaction which gradually increased during 4 or 5 weeks, after which the reaction was reversed and by the 10th week after inoculation the inoculated tubes were more alkaline than the control. The uninoculated medium changed very gradually from pH 6·9 at the beginning of the experiment to pH 6·7 in 15 weeks. Of four strains tested the lowest pH recorded was 5·8, in two others it was 6·3 and in the fourth 6·6.

Nutrient broth + 5 *per cent. saccharose.* The organism grows vigorously in this medium which has, therefore, been used for continuing the strains after isolation, transfers being taken at intervals of from 4 to 8 weeks and the cultures kept at room temperature. Such cultures tested with bromocresol purple when 2 to 3 months old almost invariably gave an acid reaction. Thus in one set of cultures using nine strains, the reaction of the cultures in 10 weeks was from pH 5·2 to 6·4 (control 7·0); in another series 5·4 to 6·6. When the cultures are incubated at 25° C. there is distinct cloudiness in 24 hours; in 2 to 3 days the medium becomes more turbid and a pellicle is produced which when disturbed may fragment, or it may fall almost whole as a stringy film. After 4 weeks, if left undisturbed, the liquid becomes clearer, but there is a whitish sediment and usually a rather firm pellicle. Cultures in this medium, whether incubated or kept at room temperature, assume a yellowish tint within a few days.

The general conclusion drawn from these tests of sugars and glycerine in broth was that with dextrose there was definite acidity produced, with lactose or glycerine no acidity, and with saccharose slight acidity followed by a change to alkalinity.

In Uschinsky's solution (synthetic medium containing glycerine) there was some cloudiness and a readily fragmenting pellicle within a few days. A yellowish coloration appeared within 10 days; this was most pronounced in the upper part of the liquid, where growth was most vigorous. In the first test with this medium the phosphate used was the basic K_2HPO_4, and the medium was found to be on the alkaline side of neutrality (about pH 7·6). When the cultures were tested with bromothymol blue at the end of a month they were found to be more acid than the controls, their reaction being about pH 6·6. In another test using KH_2PO_4 as phosphate and then neutralising with NaOH, giving a medium of pH 6·8 after sterilisation, there was found to be an alkaline reaction, the pH of the cultures finally ranging in six strains from 7·0 to 7·6.

Nutrient broth + nitrate. Tubes of nutrient broth with 0·1 per cent. potassium nitrate were inoculated and tested for nitrite, some at the end of 14 days, others in 4 weeks, using for some tubes the starch-iodide test, for others the more delicate sulphanilic acid and naphthylamine test. The results were invariably negative, indicating that the nitrate was not reduced to nitrite.

Tryptophane broth. Used as a medium to test for indol production. In this medium there was moderate growth, again with the formation of a fragile pellicle. The contents of the tubes were tested for indol by the Ehrlich-Böhme method when the cultures were 14 days old. The results were negative.

Cohn's solution. No growth was observed in this medium, except with one strain which showed a faint turbidity after 7 days.

Milk. In plain milk the upper part of the liquid shows a slight clearing within 2 days, and this decreasing opacity of the milk is quite noticeable on the 3rd day. In 12 days there is a definite layer of whey about 1 cm. deep. A soft curd is produced; this is very gradually peptonised and does not completely disappear for some months. The final result (after about 4 months) is a thick sediment, above which is a yellowish translucent liquid and at the surface a whitish ring or pellicle.

Litmus milk. In milk tinged with litmus the clearing of the milk is seen to take place in definite layers, the colour assuming a deeper hue as the opacity of the medium decreases. Usually three distinct layers can be seen within about 3 days, the colour being deepest in the uppermost layer. Gradually the litmus becomes decolorised, so that the tubes are very similar to the plain milk cultures, but later the colour may return. There is no reddening of the litmus.

Purple milk (milk tinged with bromo-cresol purple). The general result is similar to that recorded for plain milk; the indicator present shows a slight reaction in the alkaline direction.

Methylene blue milk. In this medium the colour began to fade within 2 days of inoculation, and on the third day the cultures were hardly distinguishable from control tubes. After a week's incubation the colour had completely disappeared and whey was separating out above as in plain milk. On shaking the tubes there was some return of the colour, but this soon began to fade again. Three days later, when again quite decolorised, the tubes were heated in boiling water for 2 minutes; on cooling the blue colour returned and persisted.

Nutrient agar. In poured plates of nutrient agar the surface colonies are just visible to the naked eye in 24 hours; they are about 2 mm. in

diameter within 2 days and show no very characteristic structure except that, under a low power of the microscope, faint radio-gyrose markings are visible; the embedded colonies are lenticular, while those at bottom of the medium are circular, very faint, and translucent.

Nutrient agar + 5 per cent. saccharose. This medium has been used to a considerable extent during the course of this investigation. Aderhold and Ruhland (1) made use of it during their study of the cherry organism, *Bacillus spongiosus*, and the present author found it to be a very useful medium for the isolation and preliminary identification of the shoot wilt organism. In poured plates colonies may be seen with the naked eye within 24 hours. In 48 hours the colonies show certain characteristic features, and these are looked for when new isolations are made. The plates are examined first with the naked eye, then with a hand lens (using oblique light with dark background, and also transmitted light[1]), and finally under a low power of the microscope (with a 1 in. or $\frac{2}{3}$ in. objective). The colonies assume three forms according to their situation in the medium; these are referred to here as (*a*) surface, (*b*) embedded, and (*c*) bottom colonies; the last are those which develop at the bottom of the plate where the agar is in contact with the glass.

Typically in 48 hours at 25° C. the following characters are to be seen:

(*a*) *Surface colonies.* 2–2·5 mm. diameter, pulvinate, circular and greyish white. With a lens (dark background) they appear cloudy but somewhat granulose; with transmitted light they are punctuate centrally and radial lines can usually be seen. Under the microscope they are mottled or reticulate centrally, then there are close radial lines merging into a "shaded" zone which usually shows a stippling or "wave" structure denoting an uneven surface; towards the periphery gyrose or irregular lines project into a narrow hyaline marginal zone. The margin is slightly waved.

(*b*) *Embedded colonies.* About 0·8 mm. diameter, seen with lens to be lenticular in shape, circular in surface view. Under the microscope radial lines are clearly seen when the surface of a colony is examined and they can also be made out in edge view.

(*c*) *Bottom colonies.* 1·0 to 1·2 mm. diameter, faint, finely reticulate seen with lens. Under the microscope radial lines, with interconnecting lines forming a reticulation, are clearly seen.

The above characters are best seen about the second day. As the

[1] The examination is made in front of a window and a dark sheet or curtain used as a background; for transmitted light the plate is held up to the sky. The plates are examined from the underside without removing the lids.

colonies increase in size they grow denser and the structural features become masked, but the radial lines can usually be made out for several days.

In thinly sown plates the colonies reach a diameter of about 2 cm. within a fortnight. Such colonies are rather flat but raised round the centre (umbonate). The margin is waved or provided with short flabelliform lobes.

The structure of the young colonies on nutrient agar + 5 per cent. saccharose has been carefully studied for comparison with the description of *B. spongiosus* given by Aderhold and Ruhland. Certain characters given by them for that organism did not appear on these plates, and it was thought that the discrepancies might be due to differences in the medium. The nutrient agar that has been generally used in these tests is a proprietary (Difco) brand, but for comparison a nutrient agar prepared from an extract of fresh beef was also employed. The results, however, were very similar, the close radial lines becoming resolved into gyrose lines towards the periphery of the surface colonies, the radial lines of the embedded colonies, and the reticulation of the bottom colonies again being characteristic features.

Nutrient gelatin. Stabs in nutrient gelatin result in crateriform liquefaction within 2 or 3 days. Soon afterwards the liquefaction becomes stratiform, and in a fortnight extends about 1 cm. downwards. Later the action is retarded, doubtless owing to the aërobic tendency of the organism; the gelatin in some cultures was not completely liquefied in 3 months.

In nutrient gelatin poured plates the surface colonies are 1 to 1·5 mm. in diameter in 2 days, with an irregular margin; the embedded colonies are sub-spherical but with margin irregularly dentate in optical section. By the 3rd day the surface colonies are about 5 mm. in diameter and become sunken owing to the liquefaction of the surrounding gelatin.

Nutrient gelatin + 5 per cent. saccharose. Poured plates of this medium have been prepared, using "nutrient broth" in some cases, in others beef extract as a base, the results being very similar. In 48 hours the surface colonies are about 2 mm. in diameter, irregular in shape, with a dense central region surrounded by a less dense clearer zone with very fine curled lines. The embedded colonies are sub-spherical with a dentate margin in optical section, and with faint reticulate markings. Later the surface colonies become more or less vacuolate and sunken in a shallow pit of liquefying gelatin.

Sterilised potato plugs (room temperature). There is good growth along

the streak which becomes greyish white or yellow (approximately honey yellow of Ridgway) in some cultures; this is accompanied by a bluish grey discoloration of the potato which extends right through the plug to the side not inoculated within 6 days; a distinct odour is given off.

Media containing starch. To test for action on starch cultures were prepared in nutrient broth + 0·03 per cent. rice starch (a medium used by Aderhold and Ruhland), in nutrient broth + 0·2 per cent. soluble starch, and on plates of nutrient agar + soluble starch. There was no clear evidence that the organism had any action on starch. In the liquid media, when the cultures are a week or more old, if iodine is added drop by drop, the medium is stained, but if shaken the colour rapidly disappears unless excess of iodine is added. There is at first a reddish brown colour on adding the iodine gradually which may indicate a slight action on the starch, but if excess iodine is added the liquid of the inoculated tubes is stained as deeply as that of control tubes.

Streaks were made on plates of nutrient agar + 0·2 per cent. soluble starch; when the cultures were 7 days old they were flooded with iodine. There was a slight clearing of the medium immediately below the streaks, but the starch remained unchanged in the rest of the plate.

Purple lactose agar. This medium is nutrient agar containing 1 per cent. lactose and tinted with bromo-cresol purple. In these tests it was usually employed for "stroke" cultures on slopes in test-tubes. The medium showed an alkaline reaction within 24 hours of inoculation, the blue purple colour extending into the medium around and below the stroke. In 3 to 4 days the whole of the medium from the upper end of the slope to the bottom of the butt was blue purple in colour, and there was no reversal of the reaction in cultures kept for a month or more[1].

Carrot media. The organism grows readily in carrot extract and on carrot extract agar. The carrot extract was used without adjustment of reaction and was found to be distinctly acid (pH about 5). Growing in it the organism produces turbidity and a pellicle within 2 or 3 days. If disturbed the pellicle readily fragments into flocculi, but a second and a third pellicle may be formed.

On carrot extract agar the colonies are usually first visible within 24 hours. The radial lines and gyrose markings noted for nutrient agar with saccharose are also a characteristic feature of the young surface colonies

[1] The persistence of the alkaline reaction is noted here because the bacterial canker organism, to which reference has been made in this paper, usually causes a reversal of reaction in this medium, so that eventually the whole of the medium changes from purple to yellow.

on this medium. When the cultures are about a week old the radio-gyrose markings of the surface colonies become replaced by relatively large granules, so that when examined with a lens or under the microscope the colonies appear grumose.

Saltation.

Colonies showing divergence from the type frequently occur in plate cultures of the plum wilt organism. In some strains they have appeared within about a year after isolation, in others they have not been noticed until the organism has been in culture for several years. In some cases the abnormal form has replaced the original type.

These abnormal colonies have appeared in plates of nutrient agar with sugar and of carrot agar. That they are saltations from the type is suggested by the fact that the behaviour in culture of strains derived from such colonies shows no greater difference from the type than the various normal strains show among themselves, except with regard to the structure of colonies. Moreover, the structure of these colonies on the media mentioned bears some relation to that of the type. It has been shown that the characteristic features of normal colonies on these media are close radial lines, becoming resolved into gyrose lines towards the periphery. In the saltant colonies the radial lines of the type are represented by very gyrose or curled lines, so that the radial structure is almost or completely lost. Such colonies can usually be distinguished with the naked eye by their being less dense than the type and by their somewhat irregular shape; viewed with a lens (dark background) they are more granulose or speckled.

This form has not yet been studied further, and it is not known whether it is pathogenic.

Discussion.

Several bacteria have been described as causing diseases in stone-fruit trees, viz. *B. amylovorus* (Burrill) Trev., *Ps.* (*Bacterium*) *pruni* E. F. Smith, *B. spongiosus* Aderhold and Ruhland, and *Ps. cerasi* Griffin.

The present organism differs from *B. amylovorus* chiefly in morphology (arrangement of flagella). A detailed study of *B. amylovorus*, the fire-blight organism, has not been attempted during this investigation, as it has received considerable attention by workers in America where it causes serious infection of fruit trees. Its group number as determined by Stewart (6) is 211·2322033, which is the same as that found for the

organism under consideration. Apart from their morphology cultural differences also show that the two are not identical. A culture of *B. amylovorus* was obtained from Dr E. F. Smith, and the two have been grown in parallel cultures. On nutrient agar + saccharose they have produced quite different types of colonies, while differences have been noticed in other media.

Ps. pruni is definitely a yellow organism and quite distinct from the shoot wilt organism. Rolfs (5) gave it the group number 211·2232523.

Ps. cerasi is stated to be a green organism. Griffin (2), who named and briefly described it, found morphological resemblances with *B. spongiosus*, but, in addition to its green colour, mentions certain cultural differences. Thus he writes: "I have not been able to obtain the 'vacuolated' or spongy appearing colonies in agar or gelatin containing grape sugar[1]."

B. spongiosus was named and described by Aderhold and Ruhland (1), who found it causing serious infection on branches and stems of cherry trees in Germany. The original organism seems to have been lost[2], so that direct comparison with the plum wilt organism was not possible. Aderhold and Ruhland's description of the cultural characters of *B. spongiosus* is rather meagre, except with regard to the colonies in nutrient gelatin + 5 per cent. saccharose and in nutrient agar with the same sugar. They emphasise the "spongy" structure of the colonies with the production of gum within the hollows of the "sponge." In embedded colonies of nutrient agar and sugar a characteristic feature of *B. spongiosus* is the presence of "Balken" giving a "Quallen" or jelly fish appearance to the colonies. These structures have not been seen in any of the cultures of the plum wilt organism; the characteristic markings of colonies of the latter on that medium are numerous fine radial lines, as mentioned earlier in this paper. *B. spongiosus* is also described as being stained faintly with the ordinary bacterial stains, as not curdling milk and as showing very weak growth in Uschinsky's solution, features in which it differs from the present organism. It would seem, then, that the two organisms, though perhaps closely related, are not identical.

The plum bacterial shoot wilt is caused therefore by a bacterium, which apparently has not previously been described. The name *Ps. prunicola*[3] is proposed for it. Its "Group Number" is 211·2322033, and its

[1] Aderhold and Ruhland say cane sugar ("Rohrzucker").

[2] The present writer got into communication with Prof. Ruhland who wrote to say that *B. spongiosus* had not been kept in culture.

[3] According to the recent classification adopted by the Society of American Bacteriologists its name would be *Phytomonas prunicola*.

"Index Number" showing the "Primary Characters" according to the "Description Chart" of the Society of American Bacteriologists is 5021–31100–0202.

SUMMARY

A bacterial shoot wilt, observed at the East Malling Research Station in 1923 and 1924, is described.

A bacterium isolated from infected shoots has been shown by inoculation experiments to be the cause of the disease.

The organism not only infects young green shoots, but experiments have shown that it can also cause leaf spots, and that it can induce gummosis and cankers on woody branches and on stems.

Cultural studies of the organism suggest that it has not previously been described, so the name *Ps. prunicola* is proposed for it.

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EXPLANATION OF PLATES XLIV—XLVII

PLATE XLIV.

Figs. 1 and 2. Plum bacterial shoot wilt: natural infections showing the blackened lesions on the shoots and the wilting of the parts terminal to the lesions.

PLATE XLV.

Fig. 3. A narrow lesion extending through two internodes along one side: natural infection.

Fig. 4. An early stage of infection: a young lesion on a plum shoot. × 4.

Fig. 5. An infected shoot after being kept in a moist atmosphere for 2 days. The drops of liquid were swarming with bacteria. × 4.

PLATE XLVI.

Fig. 6. A plum leaf inoculated through punctures on the right of the midrib; control punctures on the left: result 18 days after inoculation (Exp. 26/10).

Fig. 7. A plum leaf 13 days after being sprayed with a suspension (in water) of *Ps. prunicola* (Exp. 29/11).

Fig. 8. The middle plum shows two control punctures; the other two were inoculated; each inoculated spot is surrounded by a sunken discoloured area. Result 7 days after inoculation (Exp. 24/3).

Fig. 9. Plum shoot 14 days after inoculation (Exp. 25/4, shoot 2).

PLATE XLVII.

Fig. 10. The two shoots on the right were inoculated at punctures with *Ps. prunicola*; the shoots on the left were punctured but not inoculated. Result 6 days after inoculation (Exp. 25/18).

Fig. 11. Plum shoot one month after inoculation at a puncture; the tip is killed and there is a blackened lesion extending downwards through several internodes; the leaf at the lower end of the lesion has collapsed (Exp. 25/21).

(*Received May 26th*, 1930.)

Fig. 2.

Fig. 1.

WORMALD.—Bacterial Diseases of Stone-fruit Trees in Britain (pp. 725–744).

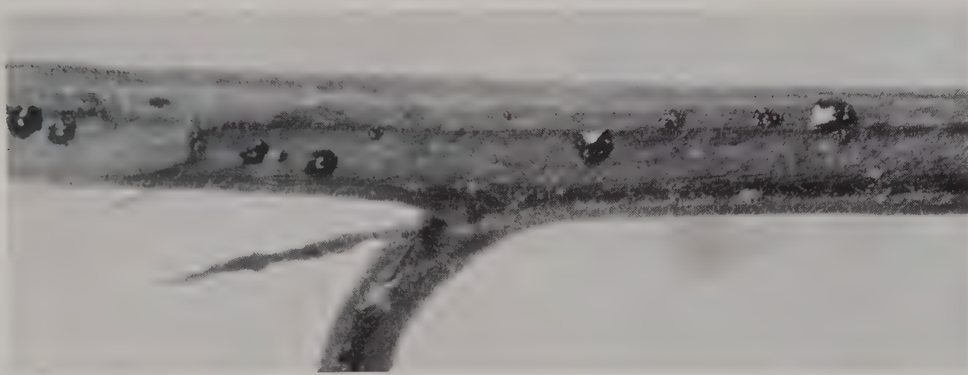

Fig. 5.

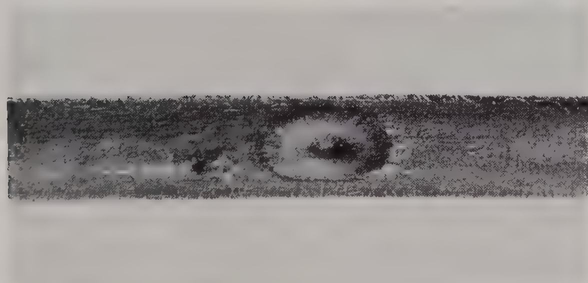

Fig. 4.

Fig. 3.

WORMALD.—BACTERIAL DISEASES OF STONE-FRUIT TREES IN BRITAIN (pp. 725–744).

Fig. 6.

Fig. 8.

Fig. 7.

Fig. 9.

WORMALD.—Bacterial Diseases of Stone-fruit Trees in Britain (pp. 725–744).

Fig. 11.

Fig. 10.

WORMALD.—Bacterial Diseases of Stone-fruit Trees in Britain (pp. 725–744).

OBSERVATIONS ON LEAF FALL IN THE DOUGLAS FIR WHEN INFECTED WITH *RHABDOCLINE PSEUDOTSUGAE* SYDOW

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(With Plate XLVIII.)

Introduction.

The needle-cast disease of the Douglas fir caused by the ascomycetous fungus *Rhabdocline Pseudotsugae* Syd. was first described by Weir (5) in America in 1917. In 1926 Wilson and Wilson (6) described an epidemic of the same disease in the South of Scotland, and the following account of the disease is essentially that given by those two investigators. There is reason to believe that the disease was introduced into Scotland prior to 1914, but it did not become serious until 1922.

In this country only two distinct varieties of the Douglas fir are commonly attacked, viz. the Blue Douglas, *Pseudotsuga glauca* Mayr, and an intermediate form which has provisionally been regarded as *Ps. Douglasii* var. *caesia* Schwerin. Of those two forms infection is most severe in the intermediate variety. The Green Douglas—*Ps. Douglasii* Carr.—is rarely attacked in this country, and only then to a very slight extent (1). The young leaves are infected during the summer and apothecia are developed in the following spring. After the spores have been shed, the infected leaves drop off the tree, *i.e.* when just over 12 months old. The exact mode of infection of the leaf has not been determined, but after it has taken place a mycelium of colourless septate hyphae is developed in the leaf, but does not pass back into the shoot. The cells of the infected area die, the contents become brown and there is a marked decrease in their starch content. About March the hyphae become more abundant towards the lower surface of the leaf, just below the two bands of stomata, and it is here that the apothecia are ultimately formed. The apothecia are purple brown and elongated, at first covered by the epidermis but later opening by a longitudinal slit, disclosing the orange-coloured hymenium, which consists of asci and paraphyses. The ascospores are mature by the

middle of May and infect the young leaves which have just emerged from the bud.

The object of this investigation was to establish as far as possible how and why needles infected with *R. Pseudotsugae* fall prematurely. The most obvious method of investigating the problem was to compare the abscission mechanism in healthy and infected leaves. The importance of physiological factors was, however, soon apparent, and in this respect several determinations were made of the water content of healthy leaves of different ages and of diseased leaves.

Anatomical study of the abscission mechanism in the Douglas fir.

The anatomy of leaf fall in the genus *Pseudotsuga* has already been shortly described by Neger and Fuchs (4), and the following, which is a somewhat more detailed account, confirms the work of these two investigators.

The anatomical structure of the abscission mechanism of the Blue, Green and intermediate variety of the Douglas fir was found to be exactly similar. The lamina of the needle joins the shoot by means of a short indistinctly defined petiole. Longitudinal sections, through the vascular bundle of the leaf base, including part of the shoot, reveal the following structures. At the point of junction of the petiole with the shoot and on the abaxial side, a single layer of thick-walled, pitted, lignified cells, containing simple crystals of calcium oxalate, stretches from just under the epidermis to the vascular bundle. On the adaxial side a similar layer of cells is present, but stop short some distance from the bundle (Plate XLVIII, fig. 1, *Lig.L.*). All the tissues distal to the lignified layer belong to the leaf and can be distinguished as follows. Adjacent to the thick-walled lignified cells, and having a similar distribution between the epidermis and the vascular bundle, is a band of comparatively thin-walled cells, two to three layers in thickness, both on the adaxial and the abaxial side of the petiole. These cells are small, densely protoplasmic, and each contains a large nucleus. The cell walls are permanently cellulosic and the epidermal cells dip markedly into the cuticle, which is thus much thinner here than at any other point. These thin-walled cellulosic cells just described constitute the true abscission layers (Plate XLVIII, fig. 1, *Ab.L.*). Distal to the abscission layers the cells are larger, thicker walled and elongated in the direction of the length of the leaf. The epidermal cells and three to six layers of sub-epidermal cells are strongly lignified (Plate XLVIII, fig. 1, *Hyp.L.*), constituting a rigid cylinder, the

strength of which accentuates the weakness of the abscission layers. On the abaxial side the endodermis is quite distinct and is completely lignified—in the lamina its outer tangential walls are not lignified. A lining layer of suberin can also be detected in the endodermal cells. A few cells, external to the endodermis on the abaxial side of the leaf base, also show more or less complete lignification, with not infrequent simple pits. On the adaxial side the endodermis is not well defined, the cell walls are thin and not lignified but suberised. Between the vascular bundle and the lignified epidermal and sub-epidermal cells on the adaxial side the cells are large, irregularly arranged, thin-walled, with a lining layer of suberin in many cases, and with scant protoplasmic contents.

On the proximal side of the thick-walled lignified cells at the base of the leaf, the cells are strongly suberised and are characterised by a greater or less infiltration of resin, which takes the lignin stains, and the presence within them of simple crystals of calcium oxalate. This constitutes a protective layer which is continuous with the stem periderm (Plate XLVIII, fig. 1, *St.Pd.*) and extends on all sides right up to the leaf trace. On the adaxial side, the phellogen is particularly active in the area between the vascular bundle and the point where the thick-walled lignified cells terminate (Plate XLVIII, fig. 1, *Pro.L.*). Thus, at the base of the petiole, there is a band of thin-walled permanently cellulosic cells, where abscission ultimately takes place, bounded above and below for most of its length by thick-walled lignified cells. Moreover, the vascular bundle is narrowest at the junction of needle and shoot.

In the above description no mention has been made of the age of the needles. All the tissues described are laid down very early in the development of the leaf, and can be seen in sections of needles taken quite near the apical bud. No further modification takes place as the needle gets older, except in the extent of development of the protective layer and the production of resin in the cells of this layer. In any individual branch the extent of the protective layer becomes proportionally greater as the leaves get older (Plate XLVIII, figs. 2, 3 and 4). But if the protective layers in leaves of the same age but from different branches on the tree are compared, it may be found that variation occurs as regards the actual amount of corky tissue comprising the protective layer. To take a particular example—two branches were obtained from the same Green Douglas fir. One, a 6-year-old lateral, was procured from fairly high up the tree. This branch bore no leaves over 5 years of age, while some of the 4-year-old leaves had already fallen. The other branch, a 10-year-old lateral, was taken from much lower down the tree, where the light

intensity was not so high. The diameter of this branch was much less than that of the other in internodes of the same age and the internodes were longer. The needles, however, remained on the branch for 8 years, but were beginning to fall in the seventh year. It was found that the protective layer in the 8-year-old needles from the shaded branch was not nearly so strongly developed as that of 5-year-old needles from the branch higher up the tree. There was also a corresponding variation in younger leaves from the two branches.

Examination of the abscission layers in the oldest leaves in mid-winter and during the spring showed, in a few cases, that the cell walls of the permanently cellulosic layers had become distinctly swollen and that the cells themselves were separating from each other, due, no doubt, to solution of the middle lamella. But these leaves were still attached to the tree, and it seems as if the needles are capable of hanging on to the tree in this condition for some considerable time.

Rupture of the cuticle and of the vascular bundle is brought about by mechanical agencies. The cuticle, however, might rupture on contraction of the tissues due to desiccation. After the leaf has fallen the open end of the vascular bundle is exposed, while the cortical tissues are protected by the band of thick-walled lignified cells and the underlying corky tissue. Some time after leaf fall the cork cambium dips down and cuts through the vascular bundle some distance from the exposed surface (Plate XLVIII, fig. 7). In this way the inner living tissues are completely protected by an unbroken periderm. The production of this phellogen, which cuts through the bundle, may be related to the entrance of air by way of the exposed surface of the vascular bundle. The cells of the occluded part of the bundle die and become filled with resin. Nothing of the nature of tylosis or blockage of the tracheids before leaf fall has been observed.

Anatomical study of infected leaves.

The mycelium of the fungus is confined to the lamina of the needle, and in no case have hyphae been observed in the petiolar region. There is absolutely nothing of the nature of an antagonism between the fungus and the host at the point of union of leaf and shoot. This differs entirely from the state of affairs that obtains in the case of spruce (*Picea excelsa*) needles infected with *Lophodernum macrosporum*. Examination of a limited amount of preserved material of diseased *P. excelsa* showed that, in this case, there is undoubtedly an antagonism between the fungus and the host at the point of union of the leaf with the leaf cushion. Just at this point there is a single layer of thick-walled cells stretching from the

epidermis right up to the vascular bundle on all sides. These cells are markedly suberised and appear to form an impassible barrier to the fungus, which is thus confined to the leaf. The cells immediately distal to this suberised layer are obviously modified by the presence of the fungus in so far as the cell walls take on a very dark colour which is visible to the naked eye as a black ring round the base of the leaf, where it joins the leaf cushion. These observations confirm the work of Neger (3) on this subject.

In the lamina of the infected Douglas fir needle the endodermis is particularly heavily infected by the fungus on the abaxial side, but hyphae have not been observed within the endodermis. Infected leaves are somewhat thinner than healthy leaves, due, no doubt, to the destruction of the protoplasm of the leaf cells, with consequent loss of turgidity. Sections were cut to see if the fungus had any effect on the thickness of the cuticle, but no difference between healthy and infected leaves of the same age could be detected.

The appearance and staining reactions of the tissues of the leaf base were exactly similar in infected and healthy leaves (Plate XLVIII, figs. 5 and 6), but infected needles fall prematurely when just over a year old. The limited extent of the fungus in the leaf and the absence of any anatomical modifications of the tissues are convincing evidence of the importance of physiological factors at work in bringing about the ultimate pathological condition of the Douglas fir host. The structure of the leaf scar is in no way different, after the needles have fallen from the normal scar, except that the corky protective layer is not so strongly developed.

The possibility of the production of toxins by the fungus, as the causal agent in defoliation, has been considered, but in the opinion of the writer this is improbable, firstly, because in the diseased leaf definite areas of infection can often be recognised between which the cells of the leaf appear to be quite normal; and secondly, because in the true abscission layers themselves the cells are in no way different from those of healthy leaves.

Water content experiments[1].

The average weight of leaves used in these determinations was about 10 gm., and in any single experiment the leaves were taken from the same lateral branch of the main shoot. The water content was determined by

[1] For these experiments the author was unable to obtain healthy material of the intermediate variety of the Douglas fir—*Ps. Douglasii* var. *caesia*—but the results show that a comparison of the water content of leaves of the same age from the Green, Blue and intermediate varieties is quite justified.

heating a known weight of leaves in an electric oven, at a constant temperature of 105° C., until successive weighings showed that all the water in the leaves had been driven off. The results of these experiments are shown in Tables I and II.

In the healthy tree (Table I) there is, on the whole, a gradual decrease in the water content of the leaves as they get older. The water content of the 1-year-old leaves is always the greatest.

Table I.

Water content of leaves from healthy trees.

Species	Location	Date of collection	Water content (%)					
			1 year	2 years	3 years	4 years	5 years	6 years
Pseudotsuga Douglasii	R.B.G.*	29. x. 28	62	60·88	59·06	57·94	—	—
,,	,,	14. xi. 28	61·89	60·61	56·22	59·96	59·88	57·78
,,	,,	6. xii. 28	64·14	63·54	61·82	61·05	60·37	58·97
Pseudotsuga glauca	Glentress Peebles	20. xi. 28	59·64	58·79	56·69	55·76	56·41	56·97
,,	R.B.G.	15. i. 29	59·79	56·84	54·78	55·62	53·85	53·63

* R.B.G. = Royal Botanic Garden, Edinburgh.

In the diseased tree it is the 1-year-old or current year's leaves that are infected and, as is shown in the first part of Table II, which presents the results obtained before the apothecia had opened, the water content of the infected leaves is distinctly lower than that of healthy leaves of the same age, whereas the water content of the older non-infected leaves from diseased trees is similar to that of leaves of the same age from healthy trees. Moreover, in the diseased trees the water content of the infected leaves is lower than that of any other leaves present on the tree.

As has been already stated, there is no obvious difference between the cuticle of healthy and diseased leaves of the same age, but sections of the infected needles used in the experiments recorded in Table II showed that the mycelium had begun to aggregate under the two bands of stomata on the underside of the leaf in preparation for the formation of apothecia, and in some instances hyphae were observed within the stomatal cavity and protruding almost to the exterior. Hyphae have not been observed in the guard cells. It may be that, after infection has proceeded so far, the stomata are no longer functional and are unable to control transpiration. Moreover, the presence of abundant hyphae in the sub-stomatal cavity greatly increases the surface area of that region, so that excessive transpiration is quite understandable.

Table II.

Water content of leaves from diseased trees before and after the fructifications have opened. (The 1-year-old needles only are infected*.)

Species	Location	Date of collection	Water content (%) 1 year	2 years	3 and 4 years	5 and 6 years	Remarks
Ps. Douglasii var. *caesia*	Glentress Peebles	4. ii. 29	52·70	Defoliated	58·32	57·24	Fructifications closed
"	"	"	52·24	"	55·12	56·65	
"	"	"	53·31	"	56·02	55·46	
"	"	"	52·74	"	54·62	55·57	
"	"	29. iii. 29	50·53	"	58·31	58·81	
"	"	"	53·14	"	56·32	56·14	
"	"	"	50·92	"	55·74	54·66	
Ps. Douglasii var. *caesia*	Glentress Peebles	24. v. 29	50·56	"	56·42	58·52	Fructifications open
"	"	"	53·33	"	57·32	59·14	
"	"	"	46·01	"	56·13	56·02	

* The material used in these experiments bore practically no 2-year-old leaves, due to the fact that the trees had been heavily infected the previous year with subsequent more or less complete defoliation of the infected growth.

Three water content determinations were made after the apothecia had burst through the epidermis, the results of which are shown in the second part of Table II. In only one case is there any appreciable drop in the water content of the infected leaves, otherwise the results are similar to those obtained before the apothecia had reached maturity.

It is interesting here to note the results of Galloway's (2) work on the leaf-cast disease of *Pinus virginiana* due to the rust fungus *Gallowaya pini* Arth. He found that before the fungus ruptured the tissues the diseased areas lost less water than the healthy portion of the leaf, due to the permanent closing of the stomata over the diseased areas. As soon as the fungus ruptured the tissues, however, evaporation increased about one-fifth above normal. In consequence of this the reserve water in the cells was gradually used up, with a subsequent loss of turgidity of the tissues. This was followed by other physiological changes which led to the gradual death and casting of the leaves. In the case of *R. Pseudotsugae*, however, the fungus does not cause the stomata to close permanently. In fact, for some time before the fungus brings about a rupture of the tissues, the mycelium is densely aggregated in the substomatal cavities, and in sections can often be seen protruding outside the stomata altogether. This suggests that the stomata are permanently open, a fact which would help to explain the marked drop in the water

content of diseased leaves, prior to the rupture of the tissues, and the absence of a further drop when the apothecia are mature.

A consideration of Table II reveals just a suggestion that the water content of healthy leaves nearest the infected areas may be affected by the presence of the disease, for in five out of the ten determinations made the water content of the 5- and 6-year-old leaves is greater than that of the 3- and 4-year-old leaves by amounts varying between 0·5 and 2·1 per cent. Many more determinations would need to be made, however, before anything conclusive could be available in this respect.

Discussion of results.

In investigating a problem such as this, there is a tendency to draw one's conclusions as to the factors at work in the abnormal, *i.e.* the diseased, host from one's knowledge of the normal. But fundamentally there is no logical reason to suppose that processes in the abnormal should not reveal, or at least give a clue to those acting in the normal host.

This study has shown that the abscission layers and associated tissues are laid down very early in the development of the leaf. No further modification takes place, except in the extent of development of the protective layer which increases proportionally as the leaves get older. It has also shown that the protective layer varies in leaves of the same age from the same tree, and that the development of the protective layer just before leaf fall is by no means constant. Hence it would appear that the extent of development of the protective layer is dependent on external growth conditions and has no causal relationship with leaf fall.

In nature the leaves do not fall in strict progression in respect to age, which suggests that leaf fall is not due to the completion of an abscission mechanism from the histological point of view. This idea is further strengthened by the fact that, in the diseased host, the infected leaves fall when just over a year old, with no abnormality of the abscission layers. Moreover, the complete defoliation of infected leaves is adequate proof that the abscission mechanism is efficient at an early stage in development of the leaf under certain physiological conditions, and this idea can probably be extended to the normal host also.

To say that the defoliation of infected leaves of the Douglas fir is ultimately due to physiological factors is practically a confession of ignorance of the causal factors involved, and the author does not pretend to think that any satisfactory explanation on physiological lines is forthcoming from this investigation. All he is prepared to say is, that the presence of *R. Pseudotsugae* brings about a marked decrease in the water

content of infected leaves, which decrease is probably of a causal nature in the ultimate pathological condition of the Douglas fir host. That there is a correlation between the water content of the leaves and leaf fall seems obvious from a consideration of Tables I and II. It has been shown (Table I) that, in the case of the healthy Douglas fir, as the leaves get older there is a gradual decrease in the water content. The possibility that this decrease is an instrumental factor in leaf fall is, if anything, strengthened by the fact that there is no strict successional defoliation in respect to age, and also by the fact that the abscission layer and associated tissues are completed at a very early stage in the development of the leaf. Also, it has been shown (Table II) that infection with *R. Pseudotsugae* brings about a marked decrease in the water content of affected leaves. Bearing in mind that it is only 5-year-old leaves that are infected, that the abscission layers and associated tissues are not invaded by the fungus, and the subsequent complete defoliation of infected leaves, it seems impossible not to admit of the intimate relationship between water content of the leaves and leaf fall.

Summary.

1. In the Douglas fir the abscission layers and associated tissues are laid down early in the development of the leaf.

2. The extent of development of the protective layer has no causal relationship with leaf fall.

3. Infection with *R. Pseudotsugae* brings about a marked decrease in the water content of infected leaves.

4. The abscission mechanism is efficient at an early stage in the life of the leaf, provided the necessary physiological conditions are present and *R. Pseudotsugae* is capable of providing these conditions.

The author wishes to express his indebtedness to Dr Malcolm Wilson for his valuable supervision of the work and criticism of the manuscript.

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EXPLANATION OF PLATE XLVIII

Fig. 1. Diagram of longitudinal section of leaf base of the Douglas fir. × 75. *Lig.L.* = Thick walled pitted lignified cells at point of union of leaf and shoot. *Ab.L.* = Permanently cellulosic layers where abscission takes place. *Hyp.L.* = Ridge of epidermal and hypodermal lignified cells. *Pro.L.* = Protective layer at point where the phellogen is most active—on the adaxial side of the vascular bundle. *St.Pd.* = Stem periderm. *End.* = Endodermis. *V.B.* = Vascular bundle.

Fig. 2. Diagram of longitudinal section of a 1-year-old leaf base of the Douglas fir. × 60.

Fig. 3. Diagram of longitudinal section of a 6-year-old leaf base of the Douglas fir. × 60.

Fig. 4. Diagram of longitudinal section of an 8-year-old leaf base of the Douglas fir. × 60.

Figs. 2, 3 and 4 were drawn from material obtained from the same branch. They show the development of the protective layer (*Pro.L.*) as the leaves get older.

Fig. 5. *Ps. Douglasii* var. *caesia*—microphotograph—longitudinal section of a 1-year-old, disease free, leaf base. × 70. Lettering as in Fig. 1.

Fig. 6. *Ps. Douglasii* var. *caesia*—microphotograph—longitudinal section of a 1-year-old leaf base infected with *R. Pseudotsugae*. × 70. Lettering as in Fig. 1.

Fig. 7. Microphotograph, longitudinal section of a leaf scar of the Douglas fir in a 5-year-old internode. × 70. *V.B.* = Occluded part of vascular bundle. *Ph.* = Phellogen.

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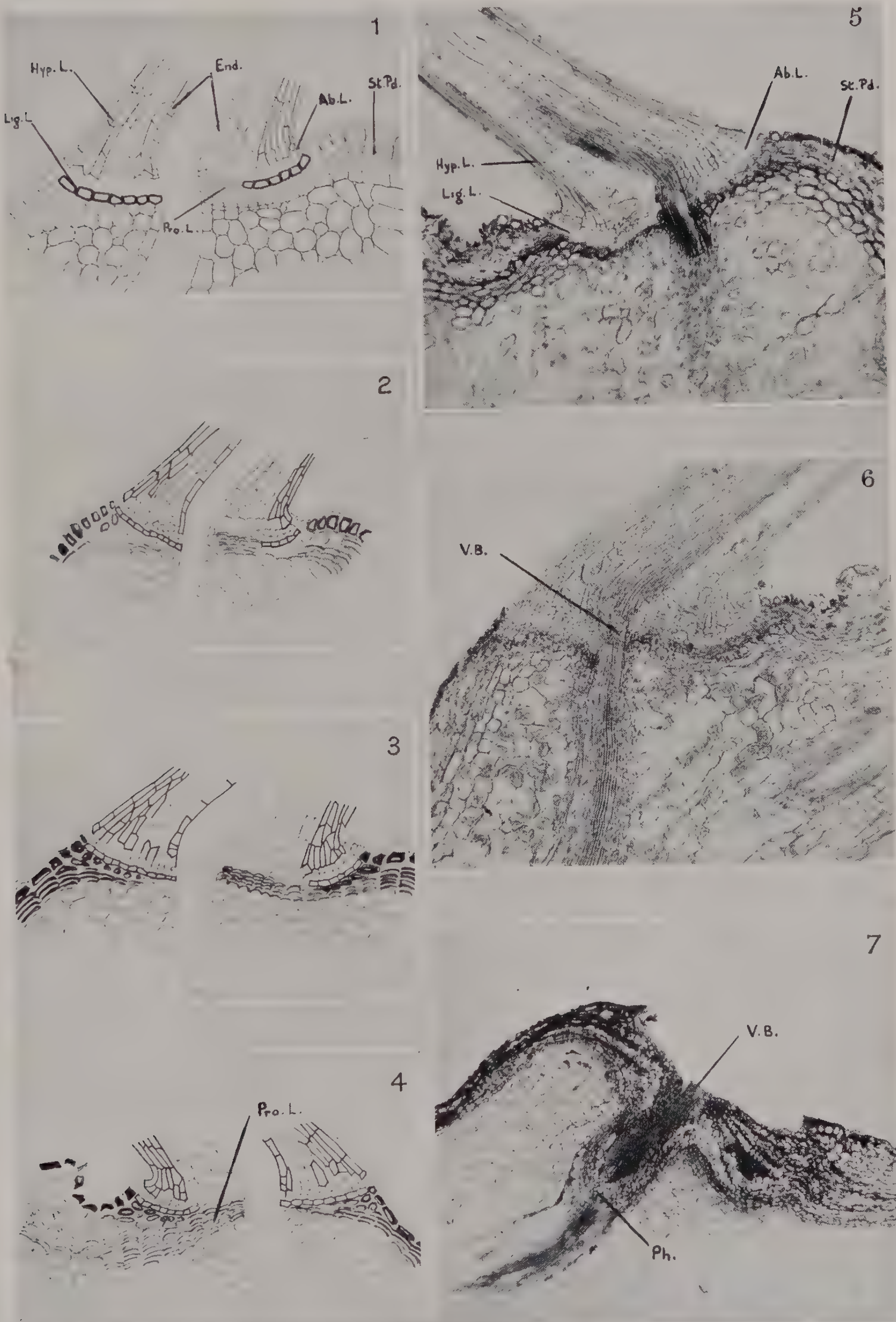

BROWN.—Observations on Leaf Fall in the Douglas Fir when infected with *Rhabdocline Pseudotsugae* Sydow (pp. 745–754).

CARBON DIOXIDE IN RELATION TO GLASSHOUSE CROPS

PART V. AN ANALYSIS OF THE RESPONSE OF THE TOMATO CROP TO AN ATMOSPHERE ENRICHED WITH CARBON DIOXIDE

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(With 3 Text-figures.)

Experiments showing that increased crops of tomatoes may be obtained by enriching the atmospheres of glasshouses with carbon dioxide emitted from a stove have been described in a previous paper (3). A glasshouse partitioned into six chambers, each 15 ft. square, and containing 56 tomato plants was used for these experiments. In those chambers in which the percentage of carbon dioxide in the atmosphere was increased, special fuel was burnt giving a concentration of 0·18 per cent. carbon dioxide or six times normal for at least $1\frac{1}{2}$ hours twice daily. Detailed observations were made in one of the chambers so treated, and in two control chambers. The method employed was to make observations of the dates of opening of the flower, pollination, ripening of the fruit, etc., of each blossom on six plants per chamber, which were marked in random positions at the beginning of the season and considered representative of the chamber. In the cases of quality of fruit and truss development additional data are available and given for all the chambers. The treated chamber gave an increase in crop of 17·1 per cent. over one control chamber and 18·0 per cent. over the other. The present paper records an analysis of this average 17·5 per cent. increase which is the result, as may be expected, of a number of components all contributing to increased yield.

A brief preliminary explanation is necessary of the terms "maturation period" and "upper and lower trusses" used in explaining the data presented. The conception of the "maturation period" is due to Bewley and Corbett (1) in whose paper a fuller description may be found.

Maturation period.

The term "maturation period" is applied to the period between the opening of the blossom and the ripening of the fruit. The average period for the fruits of different trusses of the tomato varies, being shortest for the second and third trusses. Within each truss the maturation period does not, as might be expected, lengthen gradually from the basal to the apical fruits in correlation with the opening of the blossom. It is relatively constant for the basal fruits of each truss, and there is then a well-marked interval of from 15 to 25 days during which the remaining fruits hitherto small, unswollen and thus easily distinguishable, hang and none reach the ripened stage. By the "ripened stage" is meant that at which the fruit is picked for market. It is denoted by the first appearance of the orange-red pigment of the fully ripe fruit. Finally the maturation period of these apical fruits is again relatively constant. For convenience these two classes of fruits will be termed "normal" and "retarded." The number of fruits that ripen normally or become retarded vary with the individual and with the truss.

Table I gives examples of the maturation periods found for single plants grown under normal commercial conditions.

Table I.

Maturation period in days.

Fruit	Plant 63 Truss 3	Plant 66 Truss 3	Plant 68 Truss 3	Plant 63 Truss 4	Plant 66 Truss 4	Plant 67 Truss 4
1	54	57	53	49	53	56
2	52	57	53	50	56	59
3	54	56	57	54	53	57
4	55	60	56	55	—	79
5	55	59	58	83	86	85
6	56	91	88	83	133	93
7	59	89	87	85	104	93
8	85	96	154	84	95	105
9	88	91	142	98	106	104
10	103	89	141	—	113	—
11	—	—	139	—	—	—

Table II.

Maturation period in days.

Each value is the mean of 24 plants.

Truss...	1	2	3	4	5	6	7	8	9	10
Normally ripened fruit only	55·75	55	54·75	55	60·5	66·5	64	64·5	66·5	66·25
Normal and retarded fruit	59·25	65	72·5	74·75	71·25	69	67·5	67	67·75	66·25
Difference	3·5	10	17·75	19·25	10·75	2·5	3·5	2·5	1·25	± 0

Table II shows the average maturation periods for the separate trusses calculated with and without the inclusion of the retarded fruits, the difference being a measure of the relative importance of the maturation period for different trusses.

Significance of "upper" and "lower" trusses.

By the time a tomato plant of the variety used for these experiments is maturing the fruit on the five first-formed trusses, the total amount of fruit developing is such that the next few trusses develop and set badly and yield a poor crop. It has been found that by "stopping" or cutting out the terminal bud, the period of the ripening of the five bottom trusses as a whole is shortened, presumably by the diversion of material which would otherwise have gone into the sixth and seventh trusses. Later trusses are borne on a side-shoot that takes the place of the leader. In these experiments the main shoots of the plants used were treated in this way.

The crop of a plant grown under these conditions may thus be broadly divided into that obtained from trusses 1–5 (termed the "lower trusses") and that from truss 6 onwards (termed the "upper trusses").

The greater part of the yield picked from the lower trusses is obtained from trusses 2, 3 and 4, since trusses 1 and 5 tend to be weak trusses for reasons which will be discussed later.

The crop from the upper trusses reaches maturity at a period of the season when the prices are low, and the yield from these trusses is much less, both in weight and in value, than that obtained from trusses 2, 3 and 4.

Effect of carbon dioxide on the maturation period of normal fruit.

Table IIIa and Fig. 1*a* show that the maturation period of the normal fruit of trusses 1, 2, 3 and 4 is slightly lower for the treated plants.

Table IIIa.

Maturation period in days.

Calculated omitting "retarded apical" fruit.

Truss...	1	2	3	4	5	6	7	8	9	10
Average of control chambers	55	54·5	55	54·5	60	67	64·5	64	66·5	66·5
Treated chamber	53	52	51·5	54	61	63	64	64	66	66
Days gained by treated chamber	2	2·5	3·5	0·5	−1	4	0·5	±0	0·5	0·5

Truss 3 shows the greatest average difference—3·5 days in a total period of ripening of 55 days. This may seem small, but it must be remembered that a difference of a week at this period of the year may mean a considerable difference in the price.

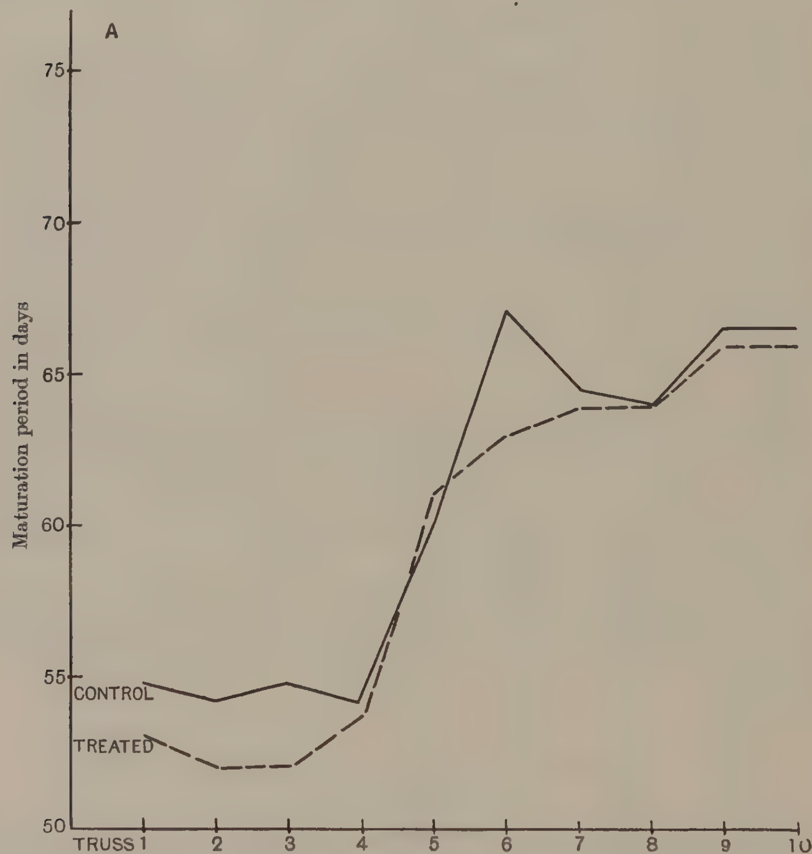

Fig. 1 *a*.

In order to test the significance of this difference, use was made of the method described by Fisher (2). Since the total crop picked from the two control chambers differed by less than 1 per cent. it is considered justifiable to treat the data from the plants in these chambers as one sample.

(1) *Truss* 3. *Maturation period of "normal" fruits* 1–5.

Untreated	$\bar{x} = 55{\cdot}0$	$n_1 + 1 = 11$	$S(x - \bar{x})^2 = 52{\cdot}5$
Treated	$\bar{y} = 51{\cdot}5$	$n_2 + 1 = 6$	$S(y - \bar{y})^2 = 8{\cdot}7$

Then $n_1 + n_2 = 15$ and $t = 3{\cdot}43$. Whence $P < 0{\cdot}01$ being beyond the limits of the table given by Fisher and the difference is clearly significant.

(2) *Truss* 2. *Maturation period of "normal" fruits* 1–7.

Untreated	$\bar{x} = 54{\cdot}5$	$n_1 + 1 = 12$	$S(x - \bar{x})^2 = 50{\cdot}5$
Treated	$\bar{y} = 52{\cdot}0$	$n_2 + 1 = 6$	$S(y - \bar{y})^2 = 16{\cdot}5$

Here $n_1 + n_2 = 16$, $t = 2{\cdot}45$, $P = 0{\cdot}03$ (approx.). Since the value of t will only be obtained by chance three times in 100 cases, this difference also must be considered significant.

Light conditions in glasshouses vary so considerably from place to place that it only becomes possible to obtain a uniform batch of plants when the area covered is a small one in the middle of a large house. In the present case, the assumption that the data derived from the six plants selected in random positions in each chamber is uniform thus implies the inclusion in the data of variation due to unavoidable variation in light conditions. Nevertheless, in spite of the inclusion of such variation, the value of t denotes a significant difference.

The difference in maturation period for the normal fruit of the upper trusses is negligible, with the exception of truss 6. The average difference of 4 days for truss 6 is obtained from less extensive data, since this truss is badly developed and the yield from it in any case is small. For the same reason it is of little value to the crop.

Effect of carbon dioxide on the maturation period of retarded fruit.

Table IIIB and Fig. 1*b* show that the differences between the truss averages for the normal fruit of the bottom trusses are increased when the retarded fruit are included in the data.

Table IIIB.

Maturation period in days.

Calculated from total fruit picked.

Truss...	1	2	3	4	5	6	7	8	9	10
Average of control chambers	59	67	73·5	76·5	70	69·5	66	67·5	68·5	66·5
Treated chamber	54	59	69	73	69	67	71	69	66	66
Days gained by treated chamber	5	8	4·5	3·5	1	2·5	−5	−1·5	2·5	0·5

The significance of this will be discussed later, together with other results. Similar data for the upper trusses are included for completeness, but, since the fruit here is borne on trusses upon shoots which grew away more rapidly in the case of the treated plants (see p. 761), ripening took

place at periods of differing weather conditions, and thus the data are not strictly comparable. Retarded fruit form only a small proportion of the crop from the upper trusses, and are of little practical importance (see Table II).

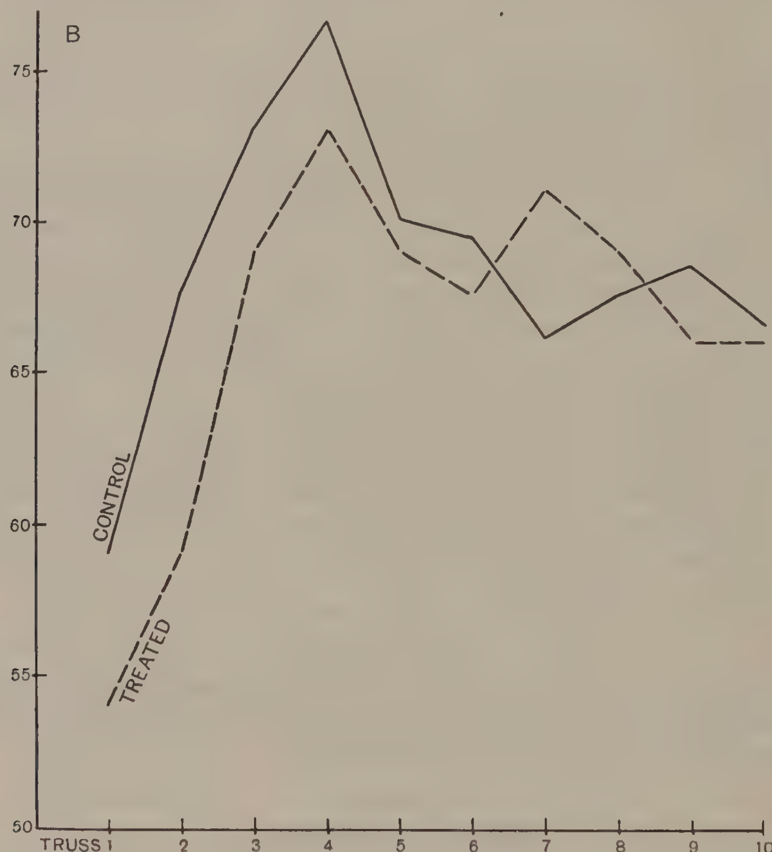

Fig. 1 *b*.

EFFECT OF CARBON DIOXIDE ON THE PROPORTION OF RETARDED FRUIT.

On trusses 2, 3, 4 and 5, which bear the greatest proportion of retarded fruit, the 12 plants of the two control chambers produced 204 "normal" fruit and 142 "retarded" fruit. The six plants of the treated chamber produced 120 "normal" fruit and 75 "retarded" fruit. The ratio $\frac{\text{normal}}{\text{retarded}}$ is thus 1·4 for the control plants and 1·6 for the treated plants. The control plants gave 69·5 retarded fruit per 100 normal fruit and the

treated plants 62·5 retarded fruit per 100 normal fruit, a difference of 7 per cent. in favour of the treated plants.

It may be suggested here that the well-defined difference in the maturation period of normal and retarded fruit, which is most noticeable at a time when trusses 1, 2, 3, 4 and 5 are loaded with fruit, is due to a shortage of nutrient material, which is sufficient for the swelling of those fruits first formed on the truss, but is then temporarily suspended until the basal fruits have ripened and been picked. It would then be expected that an increased concentration of carbon dioxide in the atmosphere will enable the plant to extend the number of fruit ripening normally further towards the end of the truss, and so eliminate some of the retarded fruits.

Effect of carbon dioxide on development of fruit on lower trusses.

Of the blossoms that are borne on a truss not all, even if pollinated, develop and reach maturity.

For the same trusses 2, 3, 4 and 5, as in the previous section, 60 per cent. of the fruit that the control plants could have borne on these trusses developed to marketable size. For the treated chamber the corresponding value is 73 per cent., a gain of 13 per cent. in favour of the treated plants.

Although temperature and humidity were automatically recorded and found not to vary in the treated and control chambers, the stoves might set up differences in air circulation. There is thus a possibility that a part of the recorded increase of 13 per cent. is due to differences in pollination conditions.

Effect of carbon dioxide on development of fruit on upper trusses.

After "stopping" of the main shoots (see p. 757), the side-shoots on the treated plants were observed to grow away more rapidly and were stronger than those of the control plants. Thus the trusses from 7 onwards of the treated plants developed and ripened earlier than the corresponding trusses of the control plants. This is shown in Table IV, giving the average dates of opening of the flower from trusses 5, 7, 8 and 9.

Table IV.

	Truss 5			Truss 7			Truss 8			Truss 9		
Chambers...	X 4	X 2	X 5	X 4	X 2	X 5	X 4	X 2	X 5	X 4	X 2	X 5
Date	11/v.	10/v.	12/v.	5/vi.	11/vi.	13/vi.	22/vi.	1/vii.	28/vi.	5/vii.	16/vii.	18/vii.
Gain in days by X 4		0			7			7·5			10	

X 2 · X 5 Control chambers
X 4 Treated chamber

Effect of carbon dioxide on development of trusses.

Observations were made throughout the season on the development of the various trusses. With the exception of the sixth truss there did not appear to be any definite differences in degree of development. Table V shows that truss 6, the lowest on the top of the plant and normally a very weak truss, is better developed on the treated plants. The figures obtained from the second, third, fifth and sixth plants of the eight plants in each row, which were considered representative of the chamber, refer only to development and not to pollination or crop afterwards picked.

Table V.

Development of the sixth truss.

	No. of trusses Very good	No. of trusses Good	Total good	No. of trusses Bad	No. of trusses Very bad	Total bad	Ratio of good and bad
X 1	4	12	16	7	5	12	1·33
X 2	4	8	12	8	8	16	0·75 (control)
X 3	8	12	20	5	3	8	2·50
X 4	7	11	18	4	6	10	1·80
X 5	3	10	13	7	8	15	0·87 (control)
X 6	9	6	15	8	5	13	1·15

Effect of carbon dioxide on the quality of fruit.

There was no difference in the quality of the fruit picked in June, July and August. The percentages of Grade A (pinks and pink and whites) and Grade B (whites) picked from each chamber throughout the season are shown in Table VI. Data are available for all the chambers; it must be noted that chambers 3 and 6 received carbon dioxide treatment for the first part of the season only. The temperature in *X* 6, an outside chamber and situated farthest from the boilers, was 3° C. colder in October than the other chambers, and must be held partly responsible for the poor swelling of the fruit in *X* 6 at the end of the season.

Table VI.

Percentages of Grade A and Grade B.

Chamber...	*X* 1		*X* 2 (Control chamber)		*X* 3		*X* 4		*X* 5 (Control chamber)		*X* 6	
Grade...	A	B	A	B	A	B	A	B	A	B	A	B
June	88	12	92	8	95	5	92	8	87	13	92	8
July	85	12	91	9	86	14	84	14	80	19	84	14
Aug.	76	19	78	19	81	16	78	20	77	21	71	17
Sept.	81	15	75	21	80	15	78	15	73	22	69	24
Oct.	80	19	62	36	76	23	72	25	72	26	65	33

The chambers given carbon dioxide treatment throughout the season produced 6 per cent. (September) and 9 per cent. (October) more fruit of Grade A (pinks, etc.) and less fruit of Grade B (whites) than the control chambers.

DISCUSSION.

The fact that the enrichment of the atmosphere with carbon dioxide has resulted in an increased yield of fruit (3) is sufficient indication that the metabolism of the plant throughout the season is influenced to a greater or lesser extent by the partial deficiency of this factor.

The next step is to enquire as to the mode of effect of an atmosphere enriched with carbon dioxide, and whether the increase is obtained steadily throughout the season or at one or more main periods.

Examination of the data given shows that the effect of an atmosphere enriched with carbon dioxide upon yield is:

(1) Small in itself in any of the following directions examined, but all contribute to a definite increase.

- (*a*) Shortening of the maturation period.
- (*b*) Increase of percentage of blossom developing into fruit.
- (*c*) Increase of proportion of "normal" fruit to "retarded" fruit.
- (*d*) Better development of sixth truss.
- (*e*) Earlier fruit-bearing at the end of the season.
- (*f*) Larger fruit at the end of the season.

(2) Not constant throughout the season but apparent at certain periods in the life of the plant.

There appear to be four such periods in the life of the plants as grown according to usual commercial practice, the plants being "stopped" above the fifth truss and a lateral allowed to grow on.

(1) The first truss is formed at a time when the young plant, with only a small area of foliage unfolded to a low light intensity, has access to an abundant supply of moisture and nutrient material. It is in a vegetative rather than reproductive phase of the life-cycle. Consequently the first truss is often poorly developed, and in commercial nurseries frequently not developed at all.

The second, third and fourth trusses, provided the vegetative stage is not unduly extended by watering or application of nitrogenous manure, develop well, and on these are borne the major portion of the crop.

(2) By the time the fifth truss is developing, the amount of fruit hanging and swelling on the second, third and fourth trusses, together with that yet unpicked from the first, is such as to become a strain on the

carbohydrate supply of the plant. Moreover, the very drastic trimming and removal of the foliage commonly practised by the grower at this time and necessitated by the light requirements of the ripening fruit, has led to a great reduction in photosynthetic area. It may be suggested that the supply of carbohydrate material to the developing fruits becomes unequal to the demand and that this strain is normally reflected in the well-defined interval of time required for the apical ("retarded") fruits to mature after the basal fruits have been picked. This then suggests an explanation of the difference in the maturation period between the basal and apical fruits of trusses 2, 3 and 4. It has been seen that there is a slight but significant difference in the maturation period of the "normal" fruits of trusses 2, 3 and 4 in favour of the treated plants, and that when the apical fruits are included in the data (Table III) this difference is accentuated. The conclusion follows that the beneficial effects of the carbon dioxide treatment are more marked upon the apical fruits which are so situated as to suffer first from a shortage of food material, etc., in a period of strain.

The fruits of truss 5 are thus maturing at a period when there are reasons for assuming that the supply of carbohydrate material becomes insufficient for the requirements of the crop. This truss tends to become imperfectly developed, and if the main shoot of the plant is continued trusses 6 and 7 are still worse situated, and may be found in some nurseries to give only a very poor yield. In the present case the main shoot was "stopped," so that trusses 6 and 7 developed on a side-shoot borne below the fifth truss.

(3) Truss 6 is formed on a young side-shoot which is still in a stage of vegetative extension similar to that of the conditions of development of truss 1. It was observed to be the only truss for which an increased concentration of carbon dioxide led to better development.

(4) Finally the fourth period comes towards the end of the season, when the plant is exhausted after continuous cropping. The effect of carbon dioxide treatment here may be seen in the better swelling of the fruit (p. 762).

These general conclusions should receive confirmation from the records of fruit picked, since, if the effects of a carbohydrate-deficiency are more marked at the periods of low fruit-production, then a supply of carbon dioxide throughout the season would tend to raise the crop to a greater extent at these periods than at others. In Fig. 2 (p. 765) the actual weights of fruit picked, reduced for comparison to the weekly percentage increase or decrease of the treated plants over the average

of the controls, are shown together with the dates of picking of each truss. It may be seen that the four periods of greatest increase (25 per cent.)

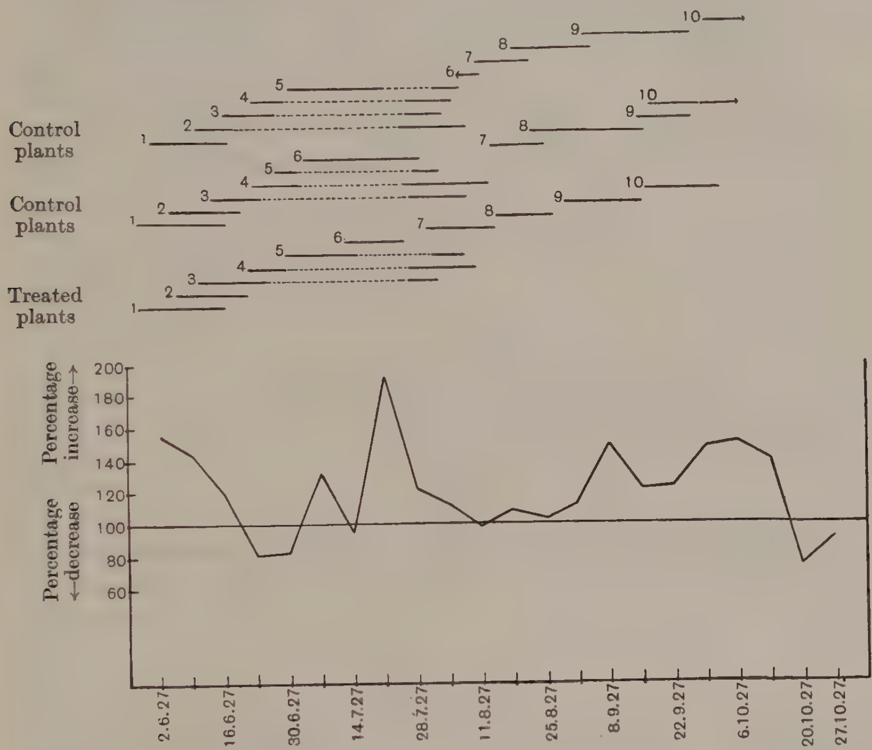

Fig. 2. The average crop from the control chambers picked each week throughout the season has been reduced to 100, and is thus represented on the diagram by a straight line. The percentage weekly deviation of the treated plants from this value is shown, together with the dates of picking of each truss plotted against time. It is thus possible to read off the separate trusses of the treated plants that show the greatest relative increase or decrease over the corresponding trusses of the control plants. The dotted lines of trusses 2–5 represent the marked differences in maturation period found for the basal and apical portions of these trusses. Earlier picking of the upper trusses of the treated plants after the main shoots have been stopped is also shown. It should be noted that the upper portion of the diagram represents the approximate dates between which the main portion of each truss was picked, and does not indicate the extent of the crop.

correspond with trusses 1, 5, 6 and from 9 onwards, which are the trusses that, as has been seen, represent periods of low fruit-production in the life of the plant.

SUMMARY.

1. Data are given showing that the process of enriching the atmosphere with carbon dioxide affects the tomato plant slightly in many different ways, the sum of these tending to an increased yield of fruit.

2. These effects include (1) shortening of the period between opening of the flower and picking of the fruit, (2) high percentage of blossom developing into fruit, (3) lower percentage of fruit retarded in ripening, (4) better development of the truss normally most poorly developed, (5) earlier development and ripening of trusses in the later part of the season, (6) better swelling of the fruit at the end of the season.

3. Conclusions are drawn from the data that the results of an increased concentration of carbon dioxide are most beneficial to the plant at periods of low fruit-production. These periods are discussed as occurring in the life of the commercially grown tomato plant.

4. The greatest percentage increases in weight of fruit of the treated plants over the controls are picked from the trusses which develop at such periods in the life of the plant.

In conclusion the writer is indebted to Dr W. F. Bewley, Director of the Cheshunt Experimental Station, and Dr F. G. Gregory, of the Imperial College, for valuable suggestions in connection with the text.

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THE BIOLOGY OF THYSANOPTERA WITH REFERENCE TO THE COTTON PLANT

VI. THE RELATION BETWEEN THE DEGREE OF INFESTATION AND THE DATE OF PLANTING

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(With 4 Text-figures.)

The relation between the date of planting a crop and the infestation of that crop by insect pests is of great importance; it is particularly important in dealing with an insect which has only one generation during the year, as it is then sometimes possible, by choosing a suitable date for planting, to insure almost complete immunity for the crop. It was not expected that the infestation of a plant by such an insect as *Thrips tabaci*, which is practically omnivorous and has several generations in one season, would show so definite a connection between the date of planting and the degree of infestation of the plant; but it did seem probable that even in this case the age of the plant when first attacked by thrips would have an important effect on the degree of infestation.

The experiments described in the following account were carried out at the Manchester University Experimental Grounds, Fallowfield, during the summer of 1929. Two types of soil were used for the experiments, a light soil (A) with hygroscopic moisture 2·53 per cent. and the loss on ignition 12·2 per cent., and a heavy clay soil (B) with hygroscopic moisture 2·60 per cent. and the loss on ignition 10·6 per cent. Sixty 10-inch pots were filled with each type of soil and divided into three blocks containing twenty pots, so that in all there were six blocks, three A 1, A 2 and A 3, containing light soil, and three, B 1, B 2 and B 3, containing clay soil. A 1 and B 1 were sown with American (Webber) cotton seed on March 28th, 1929; A 2 and B 2 were sown with the same seed on April 23rd and A 3 and B 3 on May 20th. In preceding years *T. tabaci* had first appeared about the beginning of May, so it was thought that by choosing these dates for planting the seed, blocks A 1 and B 1 should be fairly well-grown by the time the thrips appeared; blocks A 2 and B 2

should be well up and at least the second leaves should have come out, while blocks *A* 3 and *B* 3 should not germinate until the thrips were properly established in the glasshouse.

All the plants received the same amount of water, 800 c.c. per pot per week, but the soil in the *A* (light soil) blocks of plants was tilled after each watering, while in the *B* (clay soil) blocks the soil in the pots was left untouched.

The mean temperature of the glasshouse during the experiments was 27° C., a much higher figure than in either of the two preceding years; in 1927 the temperature of the glasshouse was 21° C. and in 1928 it was 19° C. The mean humidity for the period of the experiments was 71 per cent., nearly the same as in 1927 and 1928.

The two blocks of plants which were sown first, *A* 1 and *B* 1, showed a marked difference in the rate of growth; the plants in *A* 1 grew much more quickly than those in the clay soil, so that when the counts of thrips began when the plants were 4 weeks old, the plants in block *A* 1 were larger and more leafy than those in block *B* 1. This difference in growth between plants grown in light soil and those in clay soil did not occur in the other two blocks.

Infestation counts of thrips were made at intervals of 7 days in the manner described in earlier papers, only larval thrips being considered.

Fig. 1 shows the weekly infestation factors for the three blocks of plants grown in light soil. In each case the counts began when the plants were 4 weeks old. The difference between the three blocks is striking; for the first nine counts *A* 1 and *A* 2 showed a very low degree of infestation by thrips, while *A* 3 (the last planted block of plants) showed a rapid increase in the number of thrips at almost every count, and by the tenth count the large infestation factor of 199 larval thrips per 100 sq. cm. of leaf surface had been reached. Soon after this the plants in this block died as a result of defoliation caused by the thrips attack. At the eighteenth count the infestation factor for *A* 1 (the block of plants sown in March) reached 191 larvae per 100 sq. cm. of leaf surface, that is, nearly as high a factor as the maximum obtained from *A* 3. The plants in block *A* 1, however, had reached the flowering stage, and the bolls were beginning to ripen by the time the infestation factor for this block rose as high as 191, and seemed to be well able to support this degree of infestation; indeed, in previous years, plants in this stage of growth have had infestation factors as high as 250 larvae per 100 sq. cm. of leaf surface without being killed by the thrips attack. The infestation of the plants in *A* 1 reached this high level suddenly after a period of relatively low

infestation, whereas the plants in A 3 showed a relatively high infestation almost from the first count. Immediately after the eighteenth count of thrips the infestation factor for the plants in A 1 again became very low;

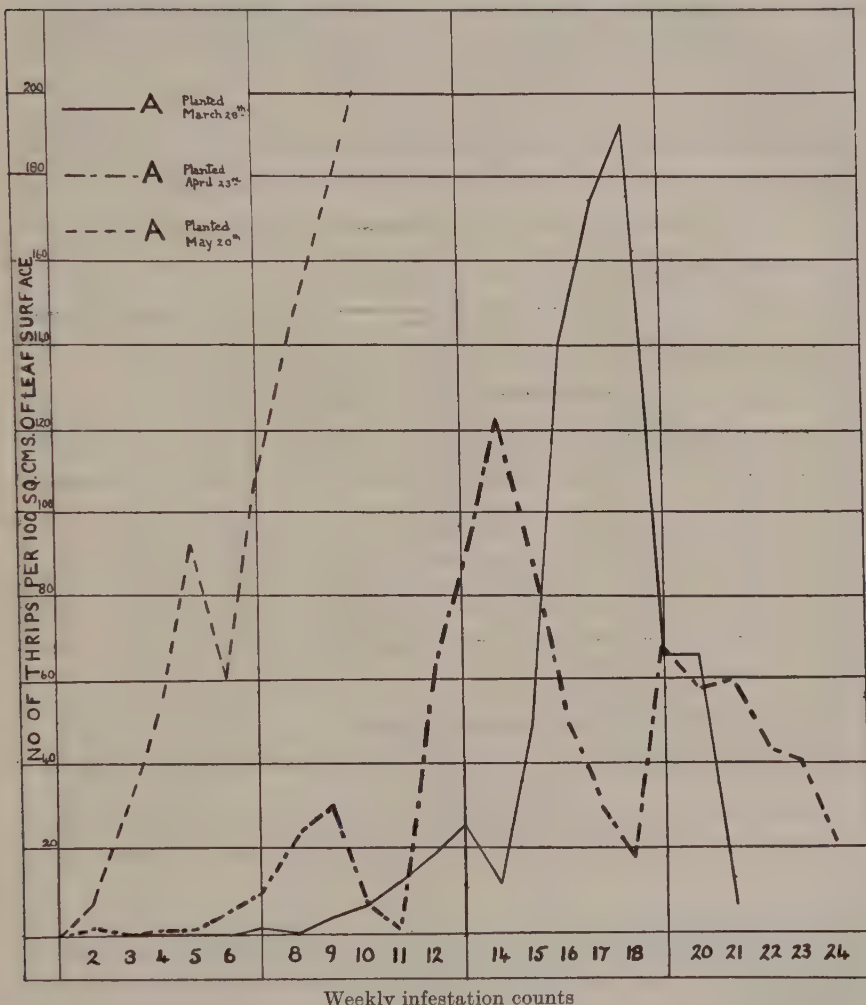


Fig. 1. The weekly infestation factors for three blocks of cotton plants, sown at different dates in light soil. In each case the counts begin when the plants are 4 weeks old.

it has been found that a particularly high infestation factor for a block of plants is frequently followed by a low one, and as a similar drop in the numbers of thrips was found on the other blocks, it almost certainly marks the end of one generation of the insects; the infestation factor for

A 3 would probably have decreased in the same way, but the plants were so badly affected that they were unable to recover. The infestation factors for *A* 1 were not taken after the twenty-first count, as by this time the plants in this block were practically over. If the graphs of the infestation of *A* 1 and *A* 2 are compared it is seen that the factors for *A* 2 were continually higher than those for *A* 1 until the fifteenth count of thrips was reached; at this point the infestation of *A* 1 began to increase rapidly. This sudden increase in the degree of infestation of *A* 1 is probably explained by the fact that, though the insects were breeding quickly and

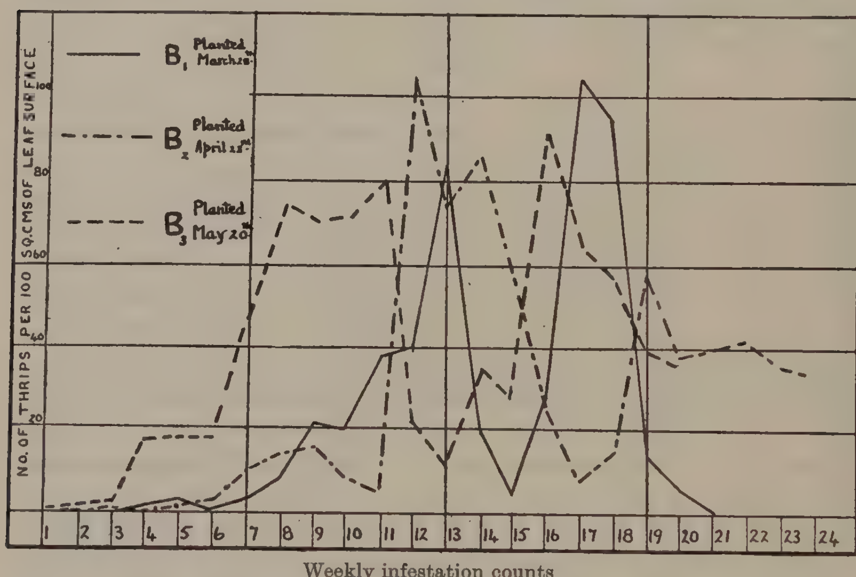


Fig. 2. The weekly infestation factors for three blocks of cotton plants sown at different dates in clay soil. In each case the counts begin when the plants are 4 weeks old.

were approaching their maximum, the plants in this block had come to the end of their vegetative phase, and were producing flowers and bolls rather than new leaves and shoots; that is, the numbers of thrips were increasing more rapidly in proportion to the increase in size of the plant than they had been doing previously. In block *A* 2, when the plants reached this stage, conditions were not so favourable and the numbers of thrips were beginning to decrease so that the infestation of *A* 2 never reached such a high level as that of *A* 1.

Fig. 2 shows the weekly infestation factors for the three blocks of plants grown in clay soil. The counts again began when the plants were

4 weeks old. The difference in the degree of infestation is not so striking as it was when the plants were grown in light soil; *B* 3 certainly shows a

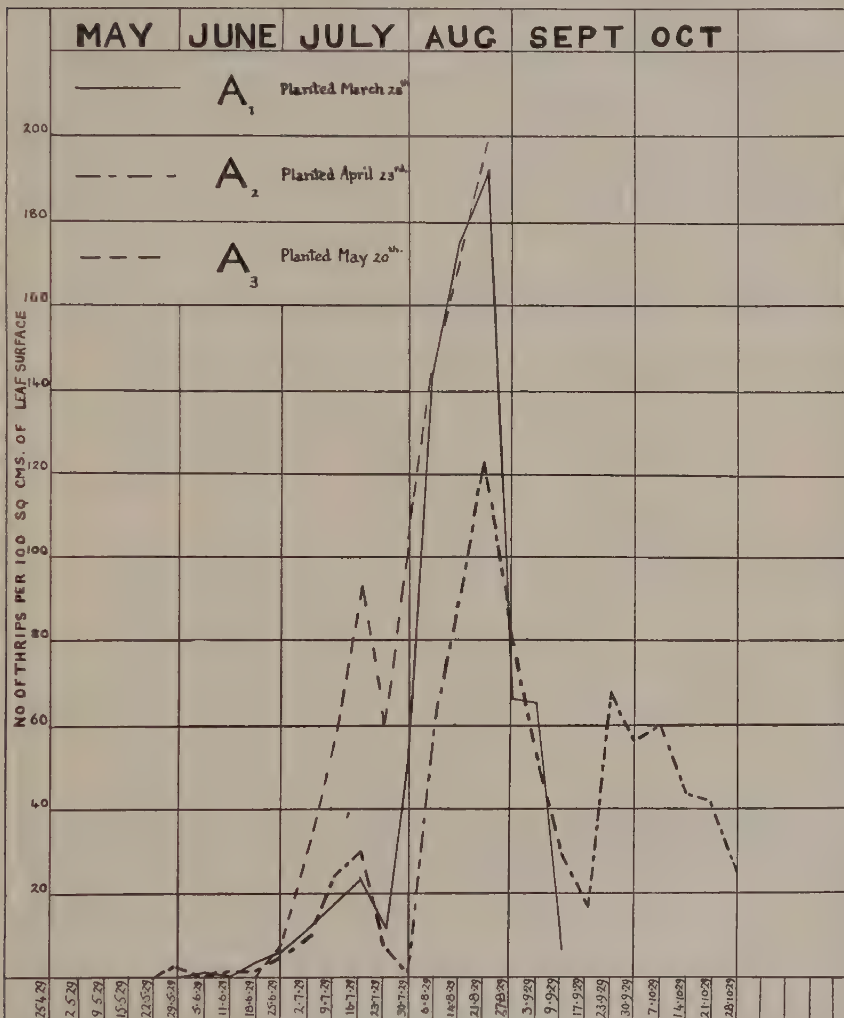
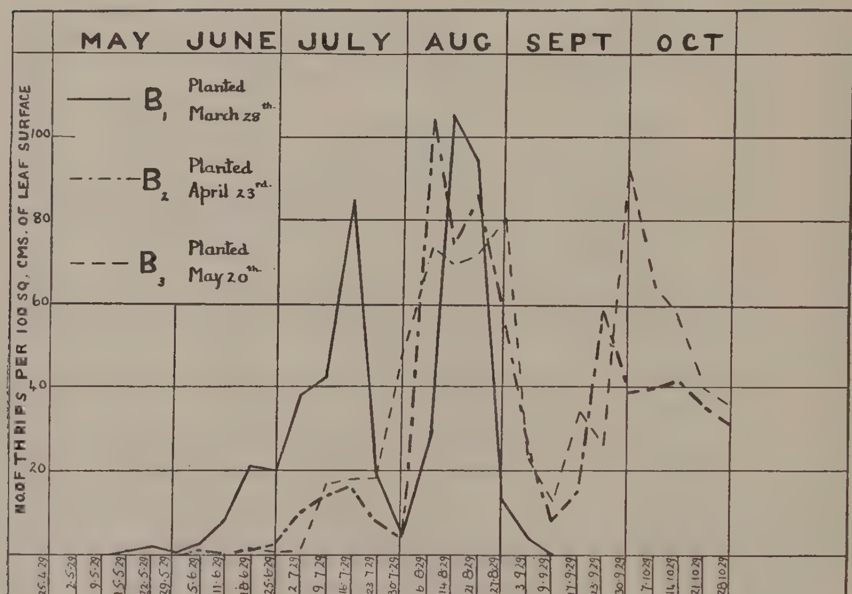


Fig. 3. The weekly infestation factors for three blocks of cotton plants sown at different dates in light soil with the actual date of each count.

much higher infestation in the early counts than the other two blocks, but the maximum number of thrips only reached 79 larvae per 100 sq. cm. of leaf surface at the eleventh count. The infestation factor for *B* 2

rose to 105 larvae per 100 sq. cm. of leaf surface at the twelfth count; this was the highest factor obtained from any of the clay soil blocks of plants, the maxima for *B* 1 and *B* 3 being 104 larvae and 92 larvae. The highest factor for *B* 3 occurred at the sixteenth count and that for *B* 1 at the seventeenth, that is, at the same time as the maximum number of thrips on the corresponding block of plants in light soil (*A* 1). None of the infestation factors for the clay soil plants was as high as the maximum factors for the plants in light soil. The infestation of *B* 1 and *B* 2 was very similar; as a rule the factors for *B* 2 were a little higher. The infestation factors for *B* 1 were not taken after the twenty-first count.



Weekly infestation counts

Fig. 4. The weekly infestation factors for three blocks of cotton plants sown at different dates in clay soil with the actual date of each count.

Fig. 3 shows the infestation of the three blocks of plants in light soil, giving the actual dates of the infestation counts without considering the relative ages of the plants; it shows that the maximum number of insects occurred about the same date on all three blocks of cotton plants.

Fig. 4 gives the infestation factors for the three clay soil blocks of plants with the date of each count. The maximum numbers of thrips on *B* 1 and *B* 2 occurred about the same date, a little earlier than the maxima for the *A* blocks of cotton plants. On *B* 3 the maximum number

of thrips was not reached until the end of September, about 6 weeks later than on any of the other blocks.

Figs. 3 and 4 point to the conclusion that, as previous experiments have shown, light tilled soil is more favourable to thrips development than clay soil, and that the plants grown in the former are likely to be more heavily infested by thrips, as in none of the blocks of plants in clay soil does the degree of infestation reach such a high level as in the corresponding block in light soil. From these figures it is seen that, in each case, the curve of the infestation factors of a block of plants can be divided into several parts separated from each other by low infestation factors, which roughly represent succeeding generations of thrips.

The mean infestation factors for the six blocks of cotton plants were:

Sown March:	*A* 1, 36·7	larval thrips per	100 sq. cm.	of leaf surface
	B 1, 23·1	,,	,,	,,
Sown April:	*A* 2, 36·6	,,	,,	,,
	B 2, 24·9	,,	,,	,,
Sown May:	*A* 3, 86·6	,,	,,	,,
	B 3, 39·2	,,	,,	,,

In each case the block of plants grown in light tilled soil shows a definitely higher mean infestation factor than the corresponding block in clay soil.

In conclusion, the preceding experiments show that the infestation by *T. tabaci* of cotton plants grown in light soil is definitely greater when the plants are sown late in the season. Late in the season the infestation of *A* 1 (the plants sown in March) rose almost as high as that of *A* 3 (sown May), but this high degree of infestation was not reached in the case of *A* 1 until after the plants had flowered and, although the plants in block *A* 3 were killed by the thrips attack, this was probably largely due to the accumulative effect of relatively high infestation factors almost from the beginning of the counts. The mean infestation factors for *A* 1 and *A* 2 are practically the same, but Fig. 1, which gives the curves for the factors of these two blocks, clearly shows that in all the earlier counts the infestation of *A* 2 was considerably higher than that of *A* 1, and it was not until the plants in the latter block had reached the flowering stage that the infestation of *A* 1 exceeded that of *A* 2.

The date at which the plants are sown does not seem to be of such great importance when plants grown in clay soil are considered. Certainly the latest planted block of plants (*B* 3) was the most heavily infested by *T. tabaci* of the three blocks grown in clay soil during the earlier counts; but the infestation of this block, although higher than that of the

other clay soil blocks, never reached such a pitch that the plants were unable to tolerate the attacks of the insects.

Summary.

Investigations were made of the degree of infestation of plants grown in light soil and in clay soil sown at different dates. The plants sown late in the season in light soil were more affected by the thrips, the infestation being relatively high almost from the germination of the plant and causing death before the flowering stage was reached. On the blocks of plants in light soil sown earlier in the year the infestation was relatively low for a considerable period, and although, at the end of the season, the thrips became very numerous on these plants it was not until after the bolls had been formed, and in this case the practical damage was small.

The plants sown in clay soil at different dates did not show such a marked difference in the degree of infestation, and all were less infested by the insects than the corresponding blocks of plants in light soil. This corroborates the findings of previous experiments, namely, that plants grown in light soil are found to be more heavily infested by *T. tabaci* than plants grown under similar conditions in clay soil.

I should like to take this opportunity of thanking Prof. Dunkerly for his helpful criticism, Miss R. M. Smith and Mr I. Thomas for their assistance in making the counts, and Mr R. Stewart for his analysis of the soils used.

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ON THE LIFE-HISTORY OF *BLASTODACNA ATRA* HAW., THE PITH MOTH OF THE APPLE

By MARY MILES.

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(With Plates XLIX–L and 3 Text-figures.)

CONTENTS.

I. INTRODUCTION.

The pith moth of the apple is a persistent pest in fruit plantations in Northern Europe, occurring commonly in England, Holland, Germany, Norway, Sweden, Denmark and Poland. Though the pest is only occasionally reported as responsible for serious loss, signs of its presence may be detected in most fruit plantations, and it is possible that the cumulative effects of slight annual attacks have been underestimated. The damage is caused by the larvae which feed beneath the bark, often eating out the entire woody tissue, and destroying vegetative shoots and blossom trusses. The injury is most apparent in the spring about the time of blossoming, when the wilted and dying tips of the attacked shoots and the shrivelling blossom trusses appear in sharp contrast with the normal leaves and flowers.

(*a*) Identity of the insect.

The pith moth is a small Tineid moth. In the revised edition of the *Handbook of British Lepidoptera*, Meyrick places the moth in the family Cosmopterygidae, but previously it had been regarded as belonging to the family Elachistidae. With regard to the generic and specific names of the insect there appears to be considerable difference of opinion. Meyrick refers to the moth as *Chrysoclista vinolentella* H.S. Throughout continental literature, however, the pith moth of the apple is referred to as *Blastodacna putripennella* Zell. In correspondence, Mr Stringer of the British Museum of Natural History quotes unpublished notes of the late Mr Hartley Durrant regarding "the true names of the apple and hawthorn *Lavernae*," concluding with the statement that "*atra* Haw. = *vinolentella* of H.S." The author hopes to deal further with the identity of the insect in a subsequent paper and, since the earliest name appears to be that of Haworth, has decided in the meantime to refer to the moth as *Blastodacna atra* Haworth.

(*b*) Methods and materials.

The occurrence of considerable numbers of the larvae of the pith moth in two fruit plantations in the vicinity of Manchester in the spring of 1928 led to a study of the life-history and habits of the insect. Damaged shoots and blossom trusses were collected and kept in the laboratory in order that observations could be made on the later stages of the life-cycle. In due course adults emerged, and these were confined in glass cylinders

so that the egg stage might be discovered. Eggs were obtained. These hatched and observations were made on the habits of the young larvae. Young larvae were transferred to a feathered maiden apple tree growing in a pot. This was kept out of doors, and periodic examinations were made to find out the habits of the larvae during the winter. Throughout the course of the investigation parallel observations were made in the field. Material collected in South Lancashire was supplemented by collections of infested twigs from a commercial fruit plantation near Hitchin, Hertfordshire.

(c) ACKNOWLEDGMENTS.

The writer wishes to acknowledge her thanks to Dr H. W. Miles for supervision and direction throughout the course of the investigation and for taking the photographs used as illustrations. She is also indebted to Prof. Balfour-Browne and Prof. Dunkerly for reading the manuscript and making helpful suggestions; to the late Dr J. Waterston, Mr H. Stringer and Mr H. Britten for identifying insects and supplying data regarding them; to Mr F. R. Petherbridge, M.A., for his interest in the investigation, and to Mr J. C. Dicker for furnishing material from time to time.

II. DESCRIPTION OF STAGES.

(a) ADULT.

The description of the pith moth of the apple, as given by Meyrick (13), is as follows:

9–11 mm. Head blackish. Fore wings narrow, blackish; plical and second discal scale tufts black, the latter anteriorly finely whitish margined; some white scales towards the costa posteriorly and apex. Hind wings dark grey.

With regard to the colour of the head of the pith moth there is much conflicting evidence. Ormerod (21) describes moths bred from larvae in apple twigs as having white heads. Theobald (29) writes: "The head is almost entirely white. It is subject to much variation. Some specimens are almost black; these Stainton considered a distinct variety." Writing some time later the same author states (37): "I have had both black and white heads," and concludes that two species of moths were present, the white-headed species, referred to as *B. hellerella* Dup., being common in Kent, and the black-headed species, referred to as *B. vinolentella* H.S., being rare in Kent and Worcester. Carpenter (1) seems to follow Meyrick

and describes the pith moth as having a black head and considers the white-headed moths a distinct species, the larvae of which live in hawthorn berries. Tullgren (40) figures the pith moth, which he names *B. putripennella* Zell., with a white head.

In the summer of 1928 the writer was concentrating her attention on the biology of the insect, and noted that among the adults there appeared some variation in the head colour, some moths having white heads, others black and others greyish. In 1929 it was decided to make careful observations on this apparent variation in the head colour. Material was collected from a number of plantations in Lancashire and Cheshire, and some was obtained from Hitchin, that from each locality being kept isolated.

When the moths emerged it was found that all were distinctly white headed. Examination under the microscope showed that the head of the moth was densely covered with white scales, among which a few darker scales occurred. Many of the moths lost some of these scales after emergence and the head then assumed a greyish or blackish appearance, depending on the extent to which the scales were removed. Re-examinations of specimens bred in 1928 showed that this tendency to lose the head scales had given rise to the differences in colour which were noted that season, and it seems possible that a similar error may have been made by other observers, particularly as the dark head coloration fitted Meyrick's (*op. cit.*) description of the moth.

The moths bred from collected material were blackish with faint light markings and prominent black scale tufts on the wings. When the moth assumed a resting position with the wings folded over the back, the light markings appeared as three pairs of whitish areas: one pair anterior to the first pair of scale tufts, the second pair at the base of the second pair of scale tufts and the third pair near the tips of the wings. In respect of these light markings the moths appear, therefore, to differ from those described by Meyrick.

When the moths were examined with a hand lens, light scales were seen mingled with the black scales on the prothorax and on the wings, where they formed a broad, faint, irregular lightish band along the posterior margins of the wings. Rusty yellow scales were mingled with the dark scales about the middle of the wings and formed an irregular rusty streak extending from the base to the tip. Stainton (27) describes the black costal blotch as "much tinged with tawny" along the inner margin. Ormerod (21) also noted the presence of the yellowish scales and wrote: "The dark part [of the wing] is more or less varied with tawny

and the light with most minute specks of black; but the great variations of colouring make it impossible to describe it serviceably." Spuler (26) considered this yellow streak one of the specific characters of the apple *Blastodacna*, but Tullgren (40) found that although this yellow coloration was distinct in some individuals it was entirely absent on others. Among the specimens which emerged in captivity during the course of this investigation the occurrence of yellowish scales among the black scales about the middle of the wing seemed constant, but there was some variation in the intensity of the yellow coloration. In some it was very faint and deeply suffused with black, while in others it was much more pronounced. Meyrick's description of the pith moth infesting the apple contains no reference to the occurrence of the yellow scales on the wings.

Mention has been made of records of variation in the appearance of the pith moth. Stainton (27) writes: "In some specimens the anterior wings are almost entirely suffused with black," and "the dark variety appears exclusively attached to apple." At the time this was written the apple and hawthorn *Blastodacnae* had not been separated and the name *Laverna atra* Haw. was used for both; the variations referred to, therefore, seem to be those separating the two species. The second quotation, borne out by an observation made the following year (28), "Among the many scores that I caught and bred from these [apple] trees, I never saw a light variety," suggests the insects attacking the apple were more or less similar in appearance. Ormerod (21), who mentions "variations of colouring," was apparently describing the moth from three specimens which had been sent to her. The scales are easily rubbed from the wings, the few flutterings of the moth in the killing bottle being frequently sufficient to remove much of the characteristic marking, therefore it is possible that the condition of her specimens accounted for such a statement as: "In the three specimens before me the right and left fore wings vary from each other in some degree in every case." Careful examination of considerable numbers of moths bred in 1929 from material collected in a variety of localities has revealed very little variation in the appearance of newly emerged moths.

(*b*) EGG.

When newly laid the eggs are a translucent pearly white. After a few days they become denser and tinged with yellow, and finally they become dull pinkish brown. They are somewhat oval in shape, about 0·5 mm. long and 0·3 mm. broad, broadly rounded at the basal end and somewhat truncate at the apex. The chorion, which is thin and easily

ruptured, is delicately sculptured by broken longitudinal ridges. These ridges form numerous small, rather pointed, projecting edges at the apex of the egg. Since the chorion never becomes hard and brittle, the egg becomes concave on the side attached to the twig while the outer surface is convex. As development takes place the head of the embryo becomes faintly discernible, appearing as a dark region near the apex towards the end of the second week of incubation.

(c) Larva.

(1) *First instar.*

Shortly after eclosion the larvae of the pith moth measure approximately 1 mm. They are transparent, greyish green caterpillars, with comparatively large dark shining heads and long setae. Metamerism is well

Fig. 1. Larva. First instar. × 70.

defined, and there are faint indications of the prothoracic and caudal plates which are a character of the later instars. The body is thickest in the thoracic region, tapers posteriorly, and is blunt and rounded at the apex. The mouth-parts are well developed, the mandibles bearing three strong teeth correlated with the habits of the young larvae. As the larvae develop they become a brownish pink colour, with prominent dark brown shiny heads, long setae and distinct brown thoracic and caudal plates.

(2) *The mature larva.*

Mature larvae measure from 7 to 8 mm. in length and are brownish pink in colour. The head is dark brown, densely chitinised dorsally and laterally, and more lightly chitinised in the frontal region and on the ventral surface. The prothorax, into which the head can be partially retracted, is covered by a brown plate, thickly chitinised round the edges, particularly posteriorly, and rather membranous towards the centre. It is broad dorsally and narrows laterally, and is divided along the median dorsal line by a narrow strip of membrane. The body tapers

caudally from the thorax, each segment having a band of deep brownish pink in the middle and gradually paling towards the intersegmental membrane. At the posterior end of the body there are three dark brown, densely chitinised caudal plates. On the eighth abdominal segment the plate is very narrow and occurs at the posterior margin. On the ninth segment the chitinous plate covers practically the entire dorsal surface, and on the tenth segment, which is broad and rounded posteriorly, the plate is roughly semicircular and bears some stout bristles. The thoracic legs are brownish and chitinised. The prolegs are pinkish at the base but transparent and membranous towards the extremities, and the caudal prolegs are brown and chitinised at the base. The planta bears a series of

Fig. 2. Mature larva. × 20.

uniordinal crotchets arranged in a curved mesoseries. The body, head and legs have numerous long setae, and the entire integument is closely covered with minute stout setulae, which may be of some service to the larva during movement under the bark or in the woody tissue of the twigs.

(*d*) PUPA.

The pupa is golden brown in colour with a dark head. It measures from 5 to 6 mm. in length, and is slender with the tip of the abdomen slightly curved forward. The wings are long and narrow, extending to the seventh abdominal segment. The antennae reach beyond the wing tips, and the extremities are in antennal cases separate from the main pupal case. The seventh and eighth abdominal segments are narrow ventrally, the eighth being very narrow. The ninth abdominal segment is broad ventrally and bears a pair of rather flattened tubular projections, the

cremaster. The arms of the cremaster point forward and upward, making an angle of rather more than 45° with the ventral surface of the body. They are flattened towards the tips, and the flat surface bears a considerable number of strong hooked bristles.

The rudimentary external generative organs of the pupae are discernible. In the male the sex character appears as a short depression lying in the median ventral line of the ninth segment, posterior to the arms of the cremaster. In the female the depression is somewhat similar in appearance, but occurs between the eighth and ninth abdominal segments, anterior to the arms of the cremaster. The position of the rudimentary genital aperture seems to be the only character separating male and female pupae.

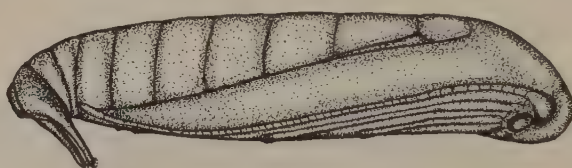

Fig. 3. Pupa. ×14.

III. BIOLOGY.

(*a*) Adult stage.

(1) *Date of emergence.*

In the laboratory moths emerged during the month of July. From material obtained from Hitchin in Hertfordshire, the period of emergence in 1928 lasted from July 9th to July 21st, while from material collected in Lancashire and Cheshire one moth emerged on July 13th, the majority of the remainder from July 17th to July 26th, and only a few specimens after that date. In 1929 moths emerged in captivity in the laboratory from July 12th to July 25th, only odd specimens emerging before and after those dates. This period of emergence seems to coincide with that obtaining out of doors. Moths were noted flying about at dusk in a fruit plantation in the vicinity of Manchester on July 25th, 1928, and were periodically observed until August 8th. Theobald (38) records the capture of pith moths at light traps during the month of July in Kent.

The length of life as an adult appears to be about 8 days in the case of males and 14 to 16 days in the case of females. Moths may, therefore, be found about the fruit plantations during the latter half of July and the early part of August.

(2) *Habits.*

During the day the moths were for the most part inactive. In the laboratory they clung to the leaves and stems of the twigs or to the muslin covering the cylinders, or remained in the bottom of the jars containing the infested material from which they had emerged. When the twigs were moved the moths continued to cling to them, making no effort to escape. On being disturbed they made short erratic flights and soon settled down again into a resting position with the wings folded close to the body. When resting on the twigs the moths were not readily discernible, since the light markings on the wings bore some resemblance to the pale lenticels and light markings on the bark.

Out of doors the moths were inactive during the day and only took to flight when the branches were shaken. At dusk, however, they became active and could be caught flying about the trees. Mating was not observed to take place during the day under laboratory conditions and was not noted out of doors.

(*b*) EGG STAGE.

Eggs appear to be laid during the night. In the laboratory, where several moths were placed in cylinders with only a few twigs, eggs were laid in the leaf axils, round the thickened bases of the leaf stalks, on the stipules of the leaves and the internodes of the stems, the majority, however, being deposited in the leaf axils. In no case were eggs found on the leaves, though this was generally accepted as the site selected for oviposition. In captivity numbers of eggs were laid about the leaf axils, but the eggs appeared to have been laid singly and were isolated on other parts of the twigs.

In the plantation the eggs of the pith moth were difficult to find. In the early part of August, 1928, three were found and their position bore out the conclusions arrived at in the laboratory: that the eggs were laid singly, near the axil of a leaf. On August 3rd, 1929, eggs were again found in the plantation, always occurring singly close to the leaf axil.

(1) *Date of egg laying and number of eggs laid.*

The date of egg laying depends upon the date of emergence of the adults, but appears to take place early in the life of the female. Eggs may, therefore, be found about the plantations during the latter part of July and the first half of August.

In order to obtain some idea of the number of eggs which might be laid, eggs were dissected from the body of a female which had died without egg laying. The abdomen was greatly distended with the egg mass, which appeared to occupy almost the entire body cavity. The eggs were enveloped in a thick mucous secretion which was dissolved in dilute alcohol and 73 fully developed eggs were counted. At the extremities of the ovarioles there were some small, incompletely developed eggs. Another female which had lived for 14 days and was known to have laid eggs, was dissected. Only six fully developed eggs remained in the abdomen, and there were no incompletely developed eggs. While recent research (6) has indicated that the female does not necessarily lay all the eggs which develop in the ovaries, some knowledge of the number developing may be useful in estimating the possible rate of increase, especially if the proportions of the sexes are known. In the material collected in various localities over two seasons the numbers of male and female pith moths have been approximately equal.

(2) *Period of incubation and time of hatching.*

In 1928 eggs which were laid in the laboratory between July 18th and July 25th commenced hatching on August 4th, indicating an incubation period of rather more than a fortnight. Two batches of eggs which had been laid during the night of July 25th hatched on August 10th, giving an incubation period of 15 days. Eggs laid July 26th–27th hatched in the laboratory on August 11th–12th after a similar incubation period. In 1929 eggs which had been laid from July 19th onwards commenced hatching on August 2nd after an incubation period of 14 days. In both seasons the laboratory was unheated and, as it has a north aspect, its temperature was often lower during the daytime than that prevailing out of doors. At other times, however, the temperature was probably higher than that out of doors.

On August 14th, 1928, two eggs were found in a fruit plantation. That they had been laid some time was apparent by their colour and the presence of the dark area at the apex, indicating the head of the developing embryo. At the same time the presence of frass and minute entrance holes about the twigs indicated that eggs had already hatched and the larvae had made their way into the plant tissue. Having regard to the time the moths emerge and the length of the incubation period, hatching probably occurs during the first half of August.

(c) LARVAL STAGE.

(1) *Observations on young larvae.*

Newly hatched larvae were kept under constant observation in the laboratory. They were active and at first wandered restlessly about the twig, apparently in search of a suitable site for feeding. After a short time, however, they settled down and commenced tunnelling into the bark, and numerous instances were noted of young larvae burrowing actively into the stems of twigs. Within about 24 hours after hatching all the young larvae appeared to have settled down to burrow into the stem tissue.

The entrance holes of the young larvae were easily discovered since they were always surrounded by fragments of rust-coloured frass. In the laboratory, where the larvae were somewhat congested, the holes occurred on practically any part of the twigs, some in the new growth where the bark was green and pubescent, and others in older wood where the bark was dark brown and tough. Some entrance holes occurred above, below, or at the side of a leaf base, while others occurred at some distance from the nodes. The occurrence of entrance holes in a variety of positions was also noted in prunings collected from a fruit plantation where infestation had been rather severe. Generally the entrance holes seem to occur in the region of a bud.

These observations on the habits of the young larvae are in direct opposition to the usually accepted beliefs. Previous writers have stated that the newly hatched larvae feed for a time on the leaves, although no evidence that they did so had been collected. Tullgren (40) in 1918 doubted the statement, often definitely asserted in literature, that the young larvae feed on the leaves during the first part of their life, and observations made in the course of this investigation justify his doubts. In the fruit plantation it was observed that the larvae had tunnelled into the bark of the twigs by the middle of August, thus establishing the accuracy of the laboratory observations. This discovery regarding the habits of the first stage larvae is of the utmost importance from the point of view of control measures, since some of the suggested measures have been based on the erroneous assumption that in the early stages the larvae of the pith moth feed on the foliage.

(2) *Larval habits and development.*

During the period August 7th–11th, 1928, newly hatched larvae were transferred to a feathered maiden apple tree growing out of doors in a pot, and in due course the characteristic entrance holes were observed in various parts of the tree.

On October 26th a twig 4 in. in length, which had been broken off by the wind, was examined. Larval entrance holes were visible with the frass still adhering to the pubescence. One entrance hole was situated near a bud which was alive but surrounded by a cankered area. On peeling away the bark a caterpillar was found. It measured nearly 3 mm., and was of a brownish pink colour with a dark head, brown thoracic and caudal plates, and long setae arising from the dorsal and lateral surfaces of the body. It was active and spun long silken threads over which it moved. It had fed over about a half-inch square of plant tissue, partly immediately under the bark where the tissue was rusty brown and partly in the woody tissue where the tunnelling was filled with frass.

A second larva, 4 mm. long, was found in a tunnel in the centre of the stem, and the terminal bud and the bud near which the larva had entered were killed. Apparently it had recently moulted, as a head capsule was found in the tunnel near it.

On November 29th a larva 3·5 mm. long was found in a piece of twig. An entrance hole was visible near the side of a bud. The larva had tunnelled towards the bud, eaten out a cavity under the bark and partly behind the bud, but without damaging it. When found, the lárva was feeding with its head turned from the tip of the shoot.

On December 20th it was noted that numbers of the tips of the shoots had been killed by the feeding of the larvae and that these dead tips were easily broken off by the wind. In one twig examined the tip was dead for a distance of 3 in. and a larva was found feeding behind a bud at the base of the dead portion. It had eaten out the entire tissue behind the bud, leaving only a thin layer of bark, thus cutting off the food supply of the tip which then perished. The cavity was filled with frass and the larva was eating back towards the base of the shoot. On the same twig was another scar about an inch long and extending about half way round the twig. A larva was feeding under the bark, the tissue under the scar being brown and discoloured, but the central tissue and the bud were uninjured. These larvae were quite active and measured about 4 mm.

On January 22nd, 1929, a length of twig was cut off for examination. Frost had been severe and snow had fallen twice since the previous examination. "Cankering," due to damage by pith moth larvae, was evident near the tip of the twig for a length of about 3 in. An entrance hole was found near the corner of another scar $\frac{1}{2}$ in. long and $\frac{1}{3}$ in. wide. The tissue beneath the bark immediately surrounding the entrance hole was brown and discoloured. The larva had eaten out a shallow tunnel behind the bud, coming very near the surface just below the base. It had

then turned round and eaten out in another direction, and was found lying with its head towards the base of the twig. The caterpillar was quite active and apparently feeding in spite of the cold weather. It measured 5 mm.

After the severe frosts lasting until the middle of February the tree was taken into the laboratory. Owing to the feeding of a considerable number of larvae all the young shoots were dead, and it was evident that the tree was dying. Early in March small heaps of new frass were noted about the twigs and, later, larvae were found to be wandering about the tree. It became obvious that, as the wood died, the larvae left the sites in which they had been feeding during the winter and wandered in search of living wood. Some of these wandering larvae measured almost 7 mm. and appeared practically fully fed, while others measured only 5 mm. Some of the larvae found their way into the healthy bark of the stock, others were put in cylinders with fresh apple twigs into which they tunnelled, and some pupated prematurely in the twigs. Owing to the difficulty of keeping apple twigs healthy for a considerable period, and the rather low humidity of the laboratory, only three adults were reared from the original batch of larvae. With the emergence of these adults, however, the life-cycle had been completed under observation.

From the observations made during the winter it was apparent that the larvae fed throughout the winter, and that severe weather conditions seemed to have no noticeable effects upon them.

(3) *Observations in the plantation, Spring* 1929.

During the winter there were few signs of the activities of the pith moth larvae. Twigs could be found showing the minute entrance holes made by the young larvae with traces of frass still adhering. Towards the beginning of April, when the buds were swelling, traces of fresh frass were observed about the trees. No larvae were found wandering about the twigs, and it was not definitely ascertained whether the frass came from new entrance holes or was merely the result of increased activity on the part of larvae which had fed in those sites throughout the winter. Reh (22) (Sorauer) states that in the spring the larvae leave their winter borings and tunnel into the base of a bud or blossom truss, and Dyckerhoff (5) makes a similar statement. From observations made out of doors and in the laboratory it would appear that the larvae occasionally feed near the surface and eat out a portion of the bark. They may return to the interior of the same twig, or possibly leave the twig and re-enter at some other point, either the tip of a shoot or in an opening blossom truss where

the bark is soft and entrance is easy. It was noted, however, that when the twigs began to swell, the cankered bark around the larval entrance holes split, and larvae were seen to push out frass through these cracks. Where the shoots are killed in the course of their feeding it seems likely that the larvae will leave their original tunnels for fresh sites in which to complete their development, but from the extent of the injury and the nature of the entrance holes it seems apparent that most of the larvae feed continuously in one site, only leaving it as adults.

(*d*) Pupal stage.

Prior to pupation the fully grown larva makes its way to the surface of the twig, near a terminal or lateral bud or a little way below a blossom truss, and eats out a circular hole in the bark through which the adult can escape. Sometimes the pupa is found protruding among the flower stalks of the dead blossoms or near a bud, or the breaking off of part of the injured shoot may leave the pupa exposed.

Duration.

Owing to pupation taking place within the twig, observations on the pupal stage are difficult to make. When fully grown larvae were disturbed they crawled from the twig and, unless they were able to tunnel into a cork or some other part of the twig, they did not pupate but shrivelled and died. Observations were made by removing a small piece of bark so that part of the larva could be seen, the bark being replaced after each examination. In most cases this disturbed the larvae and caused them to leave the twig. In one case the larva remained. It pupated on June 18th and emerged as an adult on July 18th. In another instance a mature larva entered a cork on June 22nd and an adult emerged on July 25th. From these observations it appears that the duration of the pupal stage is approximately one month. Pupation seems to take place during June, mature larvae and pupae having been found in various plantations on June 12th, 13th and 14th. Towards the end of June there were very few larvae to be seen.

IV. PARASITES.

A number of hymenopterous parasites have been reared from *B. atra*.

In 1928 several large ichneumons, identified as *Pimpla inquisitor* Scop., were reared from *Blastodacna* material collected at Hitchin. The adults emerged at the same time as the pith moths. The female has a long ovipositor which permits of the penetration of the bark of the twigs

for oviposition. From a list of host species supplied by Dr Waterston, *P. inquisitor* appears practically polyphagous and attacks Lepidoptera, Coleoptera and Hymenoptera.

In 1929 large ichneumons were again collected from Hitchin material. These were identified as *Ephialtes albispiculus* Morley. The adults emerged at the same time as the host insects. The ovipositor of the female is longer than the body and allows eggs to be deposited well below the surface of the bark. This species was observed to be ectoparasitic, and as many as three larvae were found feeding on one host larva.

A small chalcid parasite, *Copidosoma woronieckae* Now., was reared in some numbers from *B. atra* larvae from Hitchin. *C. woronieckae* is polyembryonic. The adults, both male and female, emerge at the same time as the pith moths, and in one instance 17 adults were obtained from a single pith moth larva. *C. woronieckae* was first recorded and described (19) from Poland in 1924. It is interesting to note, however, that as far back as 1908 Theobald writes (33): "The only new interesting fact concerning it (the pith moth) is that...at Greenhithe, it was widely attacked by some parasitic hymenoptera, which have not yet been identified. As many as ten of these little parasites hatched from a single larva and they appeared to be very general in the plantation. They hatched out on July 10th...." Apparently this parasite was not identified as no mention of it appears in any of Theobald's later notes on the pith moth, but it seems possible that the insect may have been the one now referred to as *C. woronieckae* Now. In the summer of 1924 Mr H. W. Miles reared a *Copidosoma* from pith moth larvae collected at Kirton, near Boston, Lincolnshire, and this was identified by Dr Waterston as *C. woronieckae*.

Another chalcid reared from *Blastodacna* larvae from both Hitchin and Maghull, Lancs., has been identified as *Habrocytus* sp. Little is known of this genus and the species cannot as yet be named.

Bethylus fusicornis Jur., which was reared from pith moth caterpillars from Hitchin, is regarded by Dr Waterston as probably a true parasite.

Hermiteles areator Grav., also obtained from larvae from Hitchin, is hyperparasitic, its host being, in the opinion of Dr Waterston, *E. albispiculus*

Other hymenoptera reared from *Blastodacna* larvae include *Omorgus difformis* Gmel., *Chelonus* sp., and *Apanteles* sp. Data regarding these insects were insufficient to determine their part in the economy of *B. atra* Haw.

V. ECONOMIC IMPORTANCE.

One of the earliest references to the economic importance of the pith moth appears to be that of Stainton (28) in 1855, who quotes from a correspondent: "This is a most destructive little wretch in apple grounds, owing to the fact of its mining in the bud and in the alburnam of the bearing spur." Since that time the moth has been frequently recorded, but not usually as causing serious injury. Ormerod reports its occurrence in 1889 (20) and 1898 (21). In 1903 Theobald (29) reports that the pith moth was abnormally abundant in 1902 and did a vast amount of damage to apples over Great Britain; it was also reported to extend its ravages to pears. In 1904 (30) he reports further damage from Kent and Gloucestershire, chiefly on young stocks. In 1904 the pest was less abundant (31); in 1906 (32) it was reported from several localities in Kent; in 1907 (33) reports of the damage were more numerous than ever and the pest was apparently increasing in numbers. In 1908 (34) the pith moth was in evidence in many plantations, and in the 1909–10 season it was not so harmful as in previous years (35). In 1905 Carpenter reported the pest from Donnybrook near Dublin (1); in 1907 from Glasnevin (2), and as "not common but occurring in South County Dublin" in 1911 (3) and 1912 (4).

In more recent years the insect appears to have caused considerable damage on the continent. In 1918 Tullgren (40) reported the pith moth as a hitherto unobserved pest in Sweden, and intimated that it was undoubtedly of considerable importance under favourable conditions. The pest was reported in Denmark (7) in 1916 and 1917 as so numerous in places as to cause complete defoliation through attack on the buds, and in 1920 (8), 1922 (9) and 1923 (10) it did considerable damage. In 1916 (23) the insect occurred in two localities in Norway, and its occurrence was again reported in 1918 (24) and 1922–3 (25). It was reported from Germany in 1920 (44), and in 1926 (12) did considerable damage in various localities. In 1923 (42) the pest was noted in Poland for the first time and in 1924 (43) it was abundant. It occurred in Holland in 1925 (41).

In this country, in the *Reports of the Ministry of Agriculture*, some damage was reported in 1919 (14) in the counties of Durham, Lancashire, Lincolnshire and Cheshire. In the report for 1920–1 (15) the insect was "noted in all fruit areas as very destructive locally," the counties concerned in 1920 being Cheshire, Warwickshire, Norfolk, Cambridge, Northamptonshire, Sussex and Hampshire; and in 1921 Durham, Cheshire, Staffordshire, Norfolk, Suffolk, London, Hampshire and Worcestershire. In the report dealing with the insect pests of crops over

the period 1925–7 the pith moth is listed among the more important of plant pests (16). In 1925–6 Theobald (39) reports an outbreak of the pith moth in Kent. In 1926 (17) Petherbridge noted the occurrence of pith moth blisters on the bark of trees in West Suffolk, and in 1928 (18) that the pest was common at Wisbech. In the Manchester province in 1928 the pith moth was present in considerable numbers in two plantations, and the injury caused was quite extensive. In 1929 the pest was again serious in the same plantations, and the writer was able to collect material from six widely separated localities in Lancashire and Cheshire.

(a) Nature and extent of injury.

In the plantations the young growing shoots and fruit spurs are killed by the tunnelling of the larvae. The damage is most readily apparent in May and June when the leaves are well developed and the trees are blossoming. The leaves of the injured shoots wilt and die, and the destruction of the terminal buds results in the pushing out of laterals. Injury to the blossom trusses is generally very obvious and results in a direct reduction in the set of fruit. Attacked blossom trusses shrivel and turn brown and die before the blossoms fully open, or occasionally shortly after the fruit has set. Besides the direct damage caused by the feeding of the larvae the injury to the bark permits the access of fungi, particularly the apple canker fungus, *Nectria galligena*. In some plantations injury to the blossom trusses appears common and causes a direct loss of crop, while in other plantations the shoot injury appears the more prevalent type. Theobald notes (32) in Kent and Sussex that not only the terminal buds were attacked but mainly the "spurs."

(b) Varieties attacked.

In Lancashire the varieties infested are Golden Spire, Grosvenor, Irish Peach, and Eckinville Seedling, while in Cheshire, Gladstone, Grosvenor, Worcester Pearmain, Allington, Bismarck, and Grenadier are attacked. Bismarck and Newton Wonder were noted as susceptible to the attacks of the pith moth in Gloucestershire and Lane's Prince Albert in Sussex. In a fruit plantation in Hertfordshire, Worcester Pearmain appears to be most susceptible, while Newton Wonder is one of the most immune; in the same plantation, however, a sport of Newton Wonder is badly attacked. In Kent in one plantation Theobald reports (32) that Worcester Pearmain was attacked, but not the alternate rows of Lord Derby; again in 1910 (36), that fruit spurs and shoots of Cox's Orange Pippin were attacked. Bush apple trees are often infested and damage from the pest is often noticeable in nursery stock.

VI. DISCUSSION OF CONTROL MEASURES IN RELATION TO LIFE-HISTORY AND HABITS.

One method of controlling the pith moth, suggested by Theobald (29, 37), was the application of a lead arsenate spray in the late summer when, it was thought, the larvae were feeding on the foliage. To avoid any arsenical deposit on the fruit the spray was to be applied as soon as the fruit was harvested, a heavy dressing being recommended. This treatment does not seem to have been widely followed, possibly owing to the difficulties of application at this season, and later (34) Theobald writes that spraying against the moths "does but little good, although there was some lessening of their numbers when arsenate of lead was used in September." Tullgren (40) doubted the efficacy of this treatment, because it had not been established that the young larvae fed on the leaves. Observations in 1928 and 1929 on the habits of the young larvae indicate that the application of an arsenical spray at this time would not be likely to give satisfactory results, since the larvae make their way under the bark almost immediately on hatching, in the first half of August.

Tullgren (40) and later Landgraf (12) suggest winter spraying with 10 per cent. Carbolinium as a means of controlling the pith moth. This suggestion is worthy of consideration, since it was noted that in Lancashire, in two plantations where the insect was very injurious, no tar oil sprays were used. Against this, however, it must be stated that the pest is generally distributed throughout a commercial plantation which has been sprayed with a tar-oil spray annually for some time. From observations on the position of the larvae during the winter and the nature of the tunnels in which they feed, the efficacy of this treatment seems doubtful. At the time the spray is applied the larvae are about half grown and well sheltered in the tissue. In several cases examined the larvae were some distance from the entrance holes which were minute, and the tunnelling between the entrance hole and the larva was filled with frass. Thus, while the "creeping" character of tar-distillate winter spray might enable it to penetrate into the tunnels, it could not be relied upon to effect a marked reduction of the insects. Cracks in the cankered bark round the entrance holes and enlarged entrance holes, which are a common feature of the attacked twigs, have not been observed until after the end of March when the trees have begun active growth. At this time the penetration of an oil spray would be much easier, but because of its ill-effects on the young growing tissue, the application of a tar-oil winter wash would be quite impracticable though summer strength white oil sprays might prove useful.

The only suitable remedy so far suggested (21, 29) is that of hand picking and destroying the infested shoots. This was carried out in a plantation at Hitchin a few years ago, and was followed by a marked reduction in the degree of infestation. Where parasites occur, particularly the small polyembryonic chalcid, *C. woronieckae*, it should be possible to adopt the method of utilising natural enemies, carried out with great success in France by Decaux (11) against the apple blossom weevil, another insect feeding in a sheltered position. The infested twigs should be collected into boxes covered with gauze which would permit the escape of the parasites while retaining the host insects. Even where the parasites are large, as in the case of *P. inquisitor*, it is generally easy to separate them from the moths, since the hymenopterous parasites are usually active in the sunlight and make their way to the light, while the moths are quiescent and remain among the twigs until late in the day.

Reh (22) and Dyckerhoff (5) recommend the use of light traps during July and August for the control of the pith moth of the apple. There does not seem to be any information available regarding the efficiency of this method of control, but Theobald (38) lists the pith moth among the species he has captured at light traps in Kent in July. The use of light traps for pest control has not been fully investigated in this country, but in the case of a serious local infestation of the pith moth they might be used with advantage to reduce the numbers of moths flying about the plantations.

Since the larvae of the pith moth appear to leave dying wood, the speedy destruction of prunings seems to be important in relation to control. Examination of prunings showed that numbers of larvae were present, and it is possible that some of these might regain a position in which they could continue their development if the prunings were allowed to remain near the fruit trees.

VII. SUMMARY.

A study has been made of the life-history and habits of the pith moth of the apple.

The adults, which are described, emerge during the latter part of July.

The eggs, a description of which is included, are laid singly on the twigs, usually near the base of the buds. Incubation requires 14 to 17 days.

Immediately on hatching, in the first half of August, the larvae tunnel into the bark of the twigs, without first feeding on the leaves.

The larvae feed continuously during the winter, weather conditions having no appreciable influence on their activities.

The pupal stage lasts about a month, approximately from mid-June to mid-July.

A number of parasites have been reared from pith moth larvae. The parasites appear unevenly distributed, few occurring in the north-west.

The pith moth is widely distributed in England and Northern Europe, and under some conditions appears to be a major pest of fruit. The larvae destroy the shoots and blossom trusses, and the cracking of the bark, which follows the injury to the twigs, permits the access of fungi.

Previously recommended control measures are discussed in relation to the life-history and habits of the insect as recorded in these investigations.

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Fig. 1.

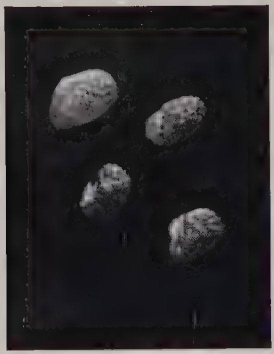

Fig. 2.

Fig. 3.

Fig. 4.

Fig. 5.

Fig. 6.

Fig. 7.

MILES.—On the Life-history of *Blastodacna atra* Haw., The Pith Moth of the Apple (pp. 775–795).

Fig. 8.

Fig. 9.

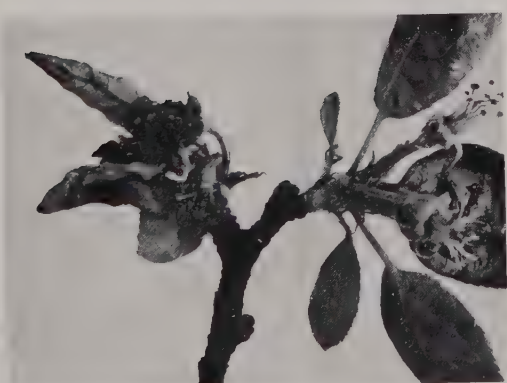

Fig. 10.

Fig. 11.

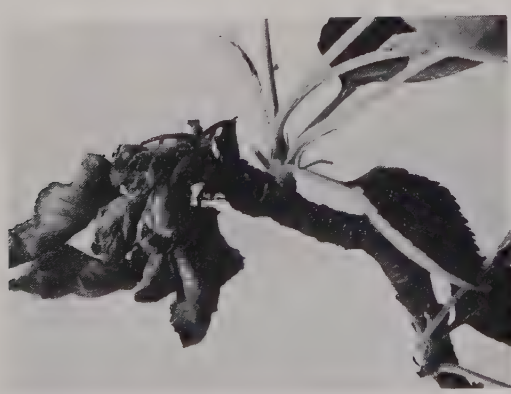

Fig. 12.

MILES.—On the Life-history of *Blastodacna atra* Haw., The Pith Moth of the Apple (pp. 775–795).

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EXPLANATION OF PLATES XLIX—L

PLATE XLIX.

Fig. 1. Eggs *in situ* at the base of a leaf. × 3.

Fig. 2. Eggs of *B. atra* Haw. × 20.

Fig. 3. Entrance holes made by newly hatched larvae in apple twig. × 3.

Fig. 4. Mature larvae. × 6.

Fig. 5. Larvae of *B. atra* parasitised by *C. woronieckae* Now.

Fig. 6. Pupae of *B. atra*. × 3.

Fig. 7. Pupa in larval tunnel in apple twig.

PLATE L.

Fig. 8. Moth at rest on apple twig, showing the white head and three pairs of white areas on the wings.

Fig. 9. Side view of moth, showing two pairs of scale tufts.

Figs. 10, 11, 12. Damage to apple twigs by the larvae of *B. atra*.

(*Received October 23rd*, 1929.)

THE PASTURE PROBLEM IN AREAS OF PERIODIC DROUGHT

By ALEXANDER NELSON, Ph.D.[1]

(*University of Edinburgh.*)

(With 1 Text-figure.)

Over wide and fairly well-defined tracts of country on the world's surface the climate follows a definite annual cycle. The major portion of the rainfall occurs during the period of the year when lower temperatures prevail. When the higher temperatures occur the rainfall is at its minimum, and in extreme cases may be nil. This cycle of "winter" rains alternating annually with summer "drought" is found typically round the shores of the Mediterranean, and is referred to as the "Mediterranean type" of climate. All the areas involved are to be found between 20° and 50° latitude both north and south of the equator. They occur always on the western side of continents, rarely, if ever, extending to the eastern coasts. The extent to which the typical cycle occurs in a continent and its occurrence in any particular district are considerably modified by a number of factors such as the incidence of mountains, occurrence of marine currents and other more or less local conditions. However, the fact does remain that large areas of the world's surface are annually subjected to regularly occurring periods of lower temperatures accompanied by higher rainfall, alternating with periods of higher temperature accompanied by lower rainfall. In the Northern Hemisphere a wide belt of such country extends from Turkestan westwards, including Palestine, the coastal regions of the Mediterranean, the north-west coast of Africa and parts of Spain and Portugal. In America a smaller area occurs approximately from the Cascade and Sierra Nevada Mountains westward to the coast, including parts of the States of Oregon, California and Arizona.

In the Southern Hemisphere there is in South America a narrow strip of coastal country from about Guayaquil in the north to about latitude 37° in the south. Another strip occurs in South Africa on the south-west and west coastal districts of the Cape Colony. In Australia a comparatively large belt occurs, extending from the west coast through the State

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of West Australia to the south coast and passing along it for a very considerable way to Adelaide at least. This belt excludes the south-west portion of the State of West Australia. The discussion which is to follow will deal with the question of the provision of stock food in these areas. It must not be assumed that the thesis to be advanced here will apply absolutely to each of these areas. For example, the temperature obtaining in the rainy season of Turkestan is very low, though the summers are very hot. Again, in the northern half of the South American belt the rainfall, though concentrated in the "winter" months, may be very small and the total may be practically negligible. All that it is proposed to do here is to indicate a theory of economic stock production, which it is believed may provide a useful line of development and research. The field observations on which the work is based were made in Australia, in greatest detail on a comparatively small piece of country in Tasmania outside the major belts defined above, yet where a cycle obtains which involves a summer drought period of varying plant stress intensity.

The matter gains its importance from economic considerations in that price rates for agricultural products are at a low ebb, and the profit margin for land is likely to recede. In addition, it is true that large commitments are now involved in land which is likely to go out of use unless either costs of production fall or returns increase. This state of affairs, acute at present, is not likely to be mitigated for some considerable time.

Whatever method or adaptation of culture may be devised for any area must, above all things, be economic. The produce must be in demand and be capable of going on to the market bearing the minimum of costs. The possibility of development of areas subject to drought by irrigation has always appealed as a positive remedy. Many parts of the areas indicated above may or may not be capable of abundant response to irrigation and so produce crops, etc., of almost any type. Irrigation, however, means in practically every case high capital expenditure with commensurate high overhead costs to be borne by the produce. This is a fundamental limiting factor in connection with irrigation, but all such schemes must be further limited in extent by such factors as the difficulties of obtaining the capital involved; the available water supply and so on. It is unlikely that pressure of world demand for food in the proximate future will render economic such large irrigation schemes as would cover a major portion of the total area cited above. In Australia, particularly, the possible labour costs involved in any attempt at fairly intensive methods of cultivation put such schemes out of practical politics at once.

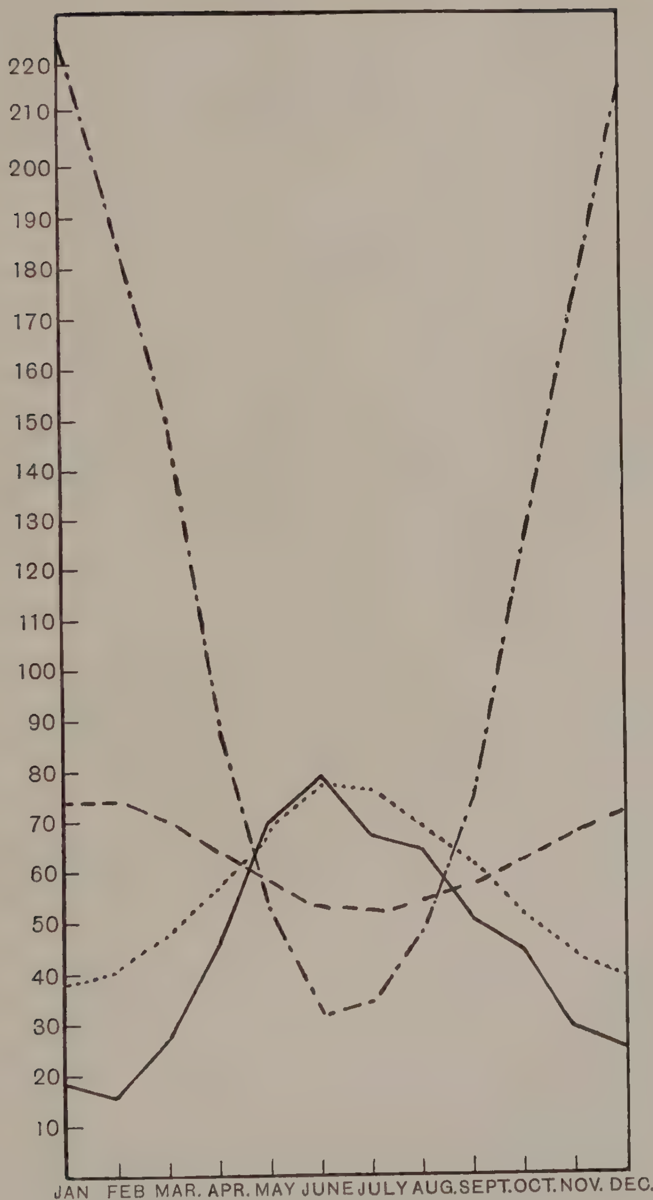
From consideration of the extent of the areas involved, some form of animal production on extensive lines is indicated, with carrying capacity of the country increased, or where stock is not already in occupation, the amelioration of the country so that they may live and do well.

The sheep, particularly in Australia, seem clearly indicated. Two economic movements in connection with sheep must be noted. In areas where closer settlement is possible and arable cultivation desirable, the production of such types of wool as cannot be borne by sheep also capable of producing marketable lamb or mutton is not now economic. Secondly, there is the fact that falling returns for wool will tend to withdraw the margin of land for wool production. The present writer is concerned here only with the second of those two movements because, if carrying capacity can be increased and drought losses minimised, land will stay in production which to-day is going out.

In brief, then, it is to the problem of increasing carrying capacity with low costs on land under a Mediterranean climate that the writer would address himself.

The problem, apart from its economic aspects, may be narrowed down to a question of the plant and its requirements. In areas where the Mediterranean type is sufficiently extreme, the plant will be faced during the year with a period when transpiration will be reasonably low due to lower temperature and higher humidity, also, at the same time, the moisture supply as by rain will be adequate for growth. Later, it will be called upon to face a period of physiological stress. Then temperatures will be high and humidity low with consequent high water loss. Over great portions of the areas cited this period of physiological stress will be sufficient to cause death of most pasture plants. At the best, there will be a period of almost nil productivity. Just what is implied by this period of stress is illustrated by the graph in Fig. 1. This shows the various climatic factors bearing on a plant in the Adelaide district. This district is by no means an extreme case, but is used because it provides accurate data on all the factors involved. The very high evaporation at the times of low rainfall and high temperature will be noted.

Vegetation subjected to extreme stress conditions such as have been postulated meets the situation in a number of ways. Species occurring naturally in the area may have been found to have adapted themselves to deal with or prevent extreme water loss, which at the moment could not be made good. Hence, more or less extreme xerophytic adaptations may occur. Again, species having developed rhizomes or other underground structures capable of weathering the stress period and propagating



Average rainfall in mm. (55 yrs.) ————
Mean daily temperature ° F. (66 yrs.) (9 h. + 15 h.) — — — —
Relative humidity per cent. (55 yrs.) 9 h. - - - - - - - - - - -
Evaporation in mm. (1870–1904) — - — - — -

Fig. 1. Climatic data for Adelaide, South Australia.

the plant, when good times return, may prove successful. Plants having a quickly maturing habit, coupled with a high rate of seed production, may be successful. Such species grow and complete their life-cycle while conditions are favourable and produce seed before the onset of the stress period. The seeds lie dormant in the ground waiting the return of the season when growth is possible.

Increased stock-carrying capacity might be attained by exploiting any of these responses of vegetation, but, in practice, all of them offer extremely difficult problems for solution. The exploitation of the drought resistant form has been attempted, but always the question of low palatability meets one, or the adaptation of the plants to resist not only drought but animal attack. The animals may thrive and do fairly well on xerophytic or semi-xerophytic vegetation, but the carrying capacity of the drought register is never high. The exploitation of this type of plant response must always be limited, first by the general unsuitability of the plants as feed and also by the comparatively high costs of establishment. It must be said, however, that once established this type of vegetation requires little or no further attention, unless a drought period of unusual severity has a lethal effect, as it might have, on only partially drought resistant plants.

Exploitation of the vegetative underground creeper is difficult. Such plants are costly to establish over large areas, in that vegetative activity seems always to go hand in hand with low seed production and, therefore, the rate of spread of such types under practically "natural" conditions is not rapid. Costs of establishment are thus rendered high. The range of species suited for employment in the class of country discussed here is small. Another drawback to the exploitation of the underground creeper is that it transfers the major portion of the food manufactured to subsurface stores out of reach of the foraging sheep. Apart from certain special areas, such as those infested by the underground grass grub in Tasmania, this method, because of high costs of establishments and comparatively low food value, precludes any likelihood of its wide adoption.

Exploitation of the seeding type of plant holds out the greatest promise for the economic increase in stock-carrying capacity of country with the Mediterranean type of climate. As is commonly recognised, pasture is the cheapest and probably is the best food for sheep. It is the only economic food under conditions where intensive methods are not practical. It may be said too that, on economic grounds, the pasture must be permanent in character. Temporary pasture, implying cultivation of the land at comparatively frequent intervals with recurring

sowings of more or less expensive seed, is ruled out for sheep husbandry in any case, and for the marginal lands under discussion it is quite impossible. Any method of pasture production developed for the areas under discussion must be low in initial cost, and require little or no outlay of labour or capital during its life, which must be long. Further, the association employed must be sufficiently palatable and nutritious to be readily eaten by sheep. The higher the carrying capacity per unit area the better. This seems an almost unsolvable problem, but a number of clues to its solution exist. Nature herself offers one clue in the well-known fact that, after a fall of rain, the drought-stricken desert is covered with vegetation. This vegetation results from the germination of seeds produced prior to the drought and lying in the dry soil awaiting moisture. The vegetation so readily produced in turn matures its seeds ready to tide the species over the next drought. The pastoralist in South Australia exploited this type of cycle in his use of subterranean clover (*Trifolium subterraneum*). To-day, over large areas in Australia, great tracts of country are being sown with subterranean clover. These are extending rapidly as the use of this annual clover becomes more widely known and, above all, as the seed becomes cheaper. The carrying capacity of land once occupied by a sparse covering of *Danthonia* is now rising rapidly following on the introduction of this plant. Each year the clover comes away rapidly with the onset of the rains, provides feed, matures seed and then dies down with the onset of the drought, to spring up again from the seed with the next year's seasonal rains. The significant point is that such pastures formed from subterranean clover are permanent owing to the seeding habit of the plant—once the initial seeding has been made, the plant through the carried-over seed regenerates the "sward" after each annual drought period. Many other species of Leguminosae and also species of Gramineae, along with feed-yielding species of other orders, behave similarly. The possibility exists, therefore, of building up an association of plants of greater diversity of food constituents and also of greater carrying capacity, and which would each year regenerate itself through the seeds lying in the ground during the drought period. Thus a method of providing sheep feed over at least a portion of the year exists. Pasture production by means of an association self-regenerating in itself as a result of a prolific seed-producing habit is cheap and fulfils practically all the economic requirements postulated earlier in the paper. Initial capital costs are low, because the price of seed of annual species, once conditions of supply and increased demand adjust themselves, is low. Very simple or no cultivation at the initial sowing is required. The whole area it is

desired to cover with such a pasture need not be and indeed, for economic reasons, should not be artificially sown. Small areas only throughout the major area to be developed should be sown and at most the seed "scratched" in. These small areas may be allowed to develop providing feed and seed and, in course of time, natural means coupled with the movement of stock will result in the spread of the introductions. Thus, from the original small colonies, the introduced seeding species will spread over the whole area; the process may be slow, but it is sure, and above all extremely economic. This gradual process of development has an added benefit in that stock carried can rise by natural increase synchronously with the rise in carrying capacity of the station, thus preventing any financial strain.

Expenses after establishment of the pasture are small, as very little after cultivation, sowing, etc., is necessary.

In Australia, however, phosphate deficiency in the soil militates against the full development of the annual plants—the clover particularly. Perkins (2) has shown that, on poor *Danthonia* country in South Australia, increased carrying capacity can easily be attained through the uses of readily available phosphate alone. Pasture analysis on the treated plots showed this increase to be due to increase in the annual "clovers" (*Trifolium* spp., *Medicago* spp.) and annual grasses, at the expense of perennial grasses and useless weeds. The whole of the increased carrying capacity following on applications of soluble phosphate may not be ascribed to soil improvement strictly, but some must be ascribed to the well-known effect of phosphate in increasing the seed-production rate of the readily responsive annuals, and possible also to increased rooting activity assisting seedling establishment and persistence. Further, phosphate may result in a hastening of maturation, so that seed is developed early, prior to the onset of the stress period. The conclusion of Richardson (3) that the addition of phosphate resulted in a more economic production of dry matter in terms of water loss is probably important in the production of these effects.

It may be, therefore, that over a great deal of the areas above cited and under discussion here, though the striking response to phosphate usual in Australia may not be forthcoming and its application may be apparently unnecessary, a small application is desirable. This, of course, will be an added cost of production, but the highly remunerative returns from "sub and super"[1] shown by the practical man in Australia do not lower the hopes of the believer in the system. In areas where response to

[1] Subterranean clover manured with superphosphate.

phosphate (or its necessity) has been demonstrated, some small amendment of the method of gradual colonisation suggested above is necessary. The phosphate would require to be applied to the initial seedlings in order to ensure a satisfactory establishment. After the first year's artificial sowing, application of the phosphate could follow the natural and animal-borne dispersal of the seed. A complete control of rise in carrying capacity and capital expenditure is thus ensured.

Exploitation of species adapted to weather periods of drought stress, by means of the naturally occurring dormant period of the seed, can thus be adapted to areas of climatic conditions, where little or nothing of feed value grows at present, or it may be superimposed on areas of similar or borderline climatic condition of present low carrying capacity—thus increasing carrying capacity on an extensive system of animal husbandry with low costs. It is possible, too, that this comparatively cheap increased carrying capacity over large areas may have the effect of checking or militating against the present serious fall in land values, which may have extremely serious effects in Australia, at least.

It is interesting to note that the small annual prolific seeding species of Leguminosae seem to be originated in Mediterranean areas, and may be regarded as an evolutionary response to the actual climatic conditions discussed here.

Certain disabilities attach to the method of self-regenerating pasture composed of annual species permanently occupying the land on account of their high rates of seed production. One is the designing of an association which will remain more or less stable from year to year. This, in itself, is not altogether serious. It is unlikely that any one year will be sufficiently extreme as to eliminate permanently any number of species united to the area. Even in a season unsuited to any one of the species, a proportion of the plants or of their seeds will be likely to persist until a season more propitious. This, in itself, provides an assurance against total failure. A more serious difficulty lies in the possibility of early rains sufficient to induce germination followed by a later short drought period resulting in the death of the vulnerable seedlings. The complete germination of all the seeds lying in the soil under such conditions is not probable, however, as many, if not all, of the species of the Leguminosae likely to be used produce a varying proportion of the super-dormant type of seed known as "hard seeds." These are most fickle in germination even under ideal conditions, and a sufficiency for continuance of the association is certainly produced under the hot, dry conditions of ripening likely to obtain under the climatic conditions postulated. The incidence of the

super-dormant seed has a beneficent bearing on the first disability mentioned above.

Perhaps the greatest disability of the self-regenerating pasture under Mediterranean type of climatic conditions is its complete cessation of feed production for a portion of the year. The fate of the stock during this period is a question of major importance. This disability may be met in a number of ways. In the case of sheep stock carried for the production of meat, intensity of stocking will synchronise with pasture production. That is, the lambs are dropped as the feed production of the pasture rises with the onset of the annual rain. This is a common phenomenon all over the pastoral areas of the world, and is the reason why lamb in New Zealand is produced in spring and summer, while lamb on the Australian mainland is produced in the winter. The whole system of economic management must turn round exploitation to the full of the flush period of the pasture. Supplementary feeding of the lambs does not usually pay, but supplementary feeding of breeding stock in the off season may and usually does pay if designed to allow of sufficient breeding stock being maintained to produce sufficient lambs to exploit the flush when it comes. Such a system of management involving fluctuating stock intensity would fit into the method of using a self-regenerating pasture as defined above. Production of lamb, however, is not likely to be widely adopted over the areas discussed. Wool production with, perhaps, meat as a by-product is more likely. Hence fluctuation of stock numbers with fluctuation in feed production would not occur to any great extent, and some method must be found of carrying the stock over the drought-stress period of low pasture production.

Supplementary feeding (provision of silage, stored grain, hay, etc.) might be considered, but, though it may be economic under certain local conditions, it is limited in its application, simply due to the high costs involved, quite apart from other reasons militating against its production.

A second possibility for getting over the drought period of low feed production lies in the migration of stock from areas where self-regenerating pastures produce cheap food seasonally to other areas where the seasonal variation in feed production does not synchronise. Such mass seasonal migrations of stock from areas of food shortage to areas of plenty are well known, and as they continue year by year must be regarded as economic. Again, however, the possibility of the exploitation of the main areas of Mediterranean climate would not be possible under such a provision and would be extremely limited where sheep were concerned. The distance travelled by stock on the hoof is limited by the capacity of

the stock and by possible loss of condition or check in growth on the one hand, and on the other by the difficulties and costs of mechanical transport.

All these methods of tiding stock over the period of drought stress and low feed productivity involve increased costs, and, apart from country on the border line climatically, are hardly practical. This would seem to negative any good accruing from the self-regenerating pasture. The practical pastoralist, however, in one area (so far limited in extent) provides the clue to an economic solution of the other half of the main problem of rendering the areas of seasonal vegetative growth productive.

On large areas of light land under a meagre seasonal rainfall (10 to 12 in.) in West Australia, a blue-flowered flat-seeded lupin, probably belonging to a variety of *Lupinus pilosus*, is carrying a large number of sheep and, in certain cases, larger stock through the summer drought period. The growing plant itself is not relished by stock, though, if allowed to graze in a paddock during the growing season, it is believed they will eat some of the lupin while feeding on the grass and other herbage between the lupin plants. Grazing of the growing lupin plant, however, is not generally advocated because the yield of the real fodder is diminished. In West Australia several sheep per acre are being held in good condition and often fattened during the summer drought period simply on the seeds and dried leaves of large areas of lupin. The variety used seems to have developed specially in the Victoria district of West Australia, and its use is spreading rapidly over a large area of light soil under the Mediterranean type of climate. The crop is self-perpetuating, and once sown the plant grows up, and sets its seed on which, along with leaves, etc., the sheep feed. Enough seed to ensure a crop when the following seasonal rains come is always trodden in by the sheep, and a system of stocking has been devised to ensure the carry over of sufficient seed. The variety used, like the smaller Leguminosae, produces hard seeds, further ensuring the carry over of the crop. Here clearly is the natural concomitant of the self-regenerating pasture—the self-perpetuating grain crop. There is no doubt that the combination of areas of self-regenerating pasture with a self-perpetuating grain crop permits of cheap animal production over vast areas of land lying under the Mediterranean type of climate. Many problems of detail require solution before the maximum exploitation of the dual method becomes possible. Species other than the lupin, no doubt, will have to be adapted to the system to suit other areas. The lupin employed in West Australia is susceptible to frost damage.

Too wide an employment of lupin species might lead to the use of one of species poisonous to stock with serious results. Species more productive or producing more nutritious food are probably available, as, for example, the Soya bean. Other problems too, such as the area of self-regenerating pasture required to balance an area of self-perpetuating grain crop, must be arrived at. Whether the pasture and the grain crop should be grown on separate areas and the sheep moved from one to the other, or whether the two should occupy the one area, may be a question. It is likely that separate areas will be involved, in that full and complete development of the grain producing crop must be ensured, but the stock, if allowed access during the growing period, would not permit this. It is important that experience tends to show that lupin alone does not provide a complete ration; some balance is required, and it is probable that this will apply to other species which may later be tried. However, once an area has been employed in this way for some time the stock will carry seeds of annual grasses and clover from the pasture to the grain crop, and the hay from the resulting plants should, after a very few years, give a mixture amongst the grain crop which will ensure a suitably balanced ration. It may be that when other species are found suited to exploitation as self-perpetuating grain crops, a number will be grown together or on separate areas, which together will provide a balanced ration.

One point of considerable importance, in connection with the system of stock management and production outlined here, is the effect the system will have on the soil. The dominance of leguminous plants amongst those used, coupled with the high incidence of animals on the area, must tend to increased and increasing fertility of the soil, both in nitrogen and organic matter. Whether this will in time lead to the displacement of the animal husbandryman by the dry farming wheat producer is one which time alone will answer. In any case, the economic practicability of the system and its soil building potentability seem undoubted. Research will be required on numerous points such as the degree to which grazing of such pastures can be carried and yet permit of sufficient seed being matured before the summer stress period sets in. The introduction of improved types of annual plants, rather than the improvement of species for drought resistance, would be required. Further, the many problems of management, both of stock and pasture and feed, will demand a considerable amount of enquiry.

It is not suggested here that stock production by the methods outlined above should compete with systems of plant culture already devised and in operation in the areas cited. All that is suggested is that areas

under Mediterranean climate which are at present not occupied with an economic crop or which are likely to go out of production altogether, owing to high costs relative to return on product, may be so saved and economic loss spared to the owner.

SUMMARY.

1. The paper deals with areas under the Mediterranean type of climate where periods of moisture supply and lower temperature with lowered evaporation alternate annually with periods of low moisture supply and higher temperature with increased evaporation.

2. The suggestion is made that in such areas where no system of economic production has already been evolved permanent pasture formation for stock production on a system of extensive husbandry would be economic.

3. That permanent pasture could be produced economically by a plant association capable of regenerating itself annually after the drought stress period owing to the production of a large number of seeds by its individual members.

4. That the stock carried by such a pasture during its production period could be carried over the alternating drought period of low or nil production by a self-perpetuating grain crop along the lines adopted in West Australia using the lupin.

5. Various difficulties and amendments of the system proposed are mentioned and discussed.

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REVIEWS

Manual of Bacterial Plant Pathogens. By CHARLOTTE ELLIOTT. Pp. ix + 349. London: Baillière, Tindall and Cox, 1930. Price 22*s.* 6*d.*

With the exception of the two works by Erwin F. Smith, *Bacteria in Relation to Plant Disease* and *Bacterial Diseases of Plants*, no book dealing solely with the phytopathogenic bacteria has previously appeared. Bacterial diseases have usually been relegated to a single, often very short, chapter in the books on plant pathology and disease-producing fungi. The outstanding exception is Stapp's contribution to the fifth edition of Sorauer's *Handbuch der Pflanzenkrankheiten* which still remains as the most adequately detailed treatment of bacterial diseases.

Until recent years plant bacteriology was the Cinderella of phytopathology, and but for the leadership of the late Erwin F. Smith it is possible that it would still occupy that position. The bacteria have always been looked upon as the particular province of the medical bacteriologists, and by them the science has been developed upon specialised lines having little direct connection with botanical problems. The methods of classification and differentiation of the bacteria, especially the differentiation by fermentation reactions, that pitfall for the unwary, and by serological methods, have nearly all been worked out by those concerned in the study of animal disease. The effect on plant bacteriology has been to relegate it to a subordinate position as a part of general plant pathology, and until recently it was not taught as a special subject in the botanical schools. In this respect it resembles the allied subject of virus diseases, which only during the last few years has received the attention which its importance merits.

Increasing recognition of the loss due to bacterial diseases impressed upon plant pathologists the need for bringing this side of the science into line, and the modern tendency is to separate bacterial pathology as a distinct branch of study with its own technique and methods of treatment. It forms in fact a link between fungal pathology and virology, on the one hand, and medical bacteriology on the other. It is inevitable that plant bacteriology should be highly coloured by, and derive many of its methods and criteria from, the older medical science, but gradually a distinct technique is being evolved.

From this point of view of plant bacteriology as a separate study the book under review meets a very definite need. The works of Smith and Stapp deal with the subject from the standpoint of the disease and the host respectively. In this new volume the author's purpose is "to place emphasis on the causal organism and to bring together in one publication a complete alphabetical list of bacterial plant pathogens and associated organisms together with their synonyms and the source and date of publication of each name." The book is divided into three parts; the first part, which forms three-quarters of the whole volume, consists of an alphabetical list of all known pathogens. The author, recognising the confusion which exists in bacterial classification, considers it "least confusing to follow the system of nomenclature under which the largest number of bacterial plant pathogens have been named." She therefore adopts Smith's modification of Migula's system, dividing the bacteria into the genera *Bacillus*, *Bacterium*, and *Aplanobacter*. It would appear however that an even less confusing method would have been to disregard generic terms and arrange the bacteria in alphabetical order of specific name throughout. This may sound unscientific, but it is doubtful if any of the present classifications can be described as "scientific," and at least it would help in ease of reference. The second part comprises a list of non-pathogenic organisms which are commonly found in association with plant diseases. Some of these were described by their discoverers as pathogens, but the evidence is inconclusive, and for this reason the author includes them with the known saprophytes.

This criticism may be, and in fact is in this book, levelled at certain of the organi listed among the pathogens. There seems, for example, to be little reason to include the *Bacillus gossypina* of Stedman or the *B. sacchari* of Janse among the pathogenic group, for these, and several others so included, have little more claim to be considered as true parasites than some of the organisms placed in the non-pathogenic group.

The third part consists of a chart of the morphological, cultural and physiological characters of the pathogens arranged in chronological order of discovery. It is difficult to see why this arrangement was chosen, for it makes reference to a particular organism much more difficult, since few workers know offhand the date of discovery of a particular organism. To have adopted the alphabetical order of the text would have made this section much more useful. It is unfortunate that the details in this chart should be so incomplete, but this is not the fault of the author.

Perhaps the most useful feature of this book is the very complete bibliography given for each organism. Notes are given concerning many of the references, and in future editions these comments and abstracts might usefully be extended, since with so exhaustive a list it is sometimes difficult to distinguish the more important references from those which contain a mere comment on the disease, or some small point in connection with it. In spite of the criticism to which this book is open, it is a valuable contribution to the field of bacterial phytopathology and should be in the hands of every research worker on the subject.

R. H. STOUGHTON.

Fungous Diseases of Plants in Agriculture, Horticulture and Forestry. By JACOB ERIKSSON. Second Edition translated from the German by WILLIAM GOODWIN. Pp. vii + 526. 399 figs. Baillière, Tindall and Cox, 1930. Price 35*s*. net.

When, twenty years ago, Eriksson first published his book on plant diseases there were very few text books available on this subject. Even the small volume was, therefore, a welcome addition to the literature, and German and English translations appeared almost immediately. For some years it has been only too heavily impressed upon the time and pockets of all working scientists that to the making of books there is no end, and the making of a new book by any author requires a great deal of justification. In 1926 and 1928 Eriksson produced the two volumes making the second edition of his *Die Pilzkrankheiten*, and now Professor Goodwin has made another book by translating these into English. This volume is the largest single book produced in this country on Fungous Diseases of Plants, and it has all the format of a reliable standard work. In spite of this it is my opinion that its translation and production were not justifiable.

The book opens with two pages on the structure and nature of fungi, followed by two chapters dealing respectively with diseases caused by bacteria and myxomycetes. In the next eighteen chapters the diseases due to fungal invasion are considered in order of classification of the fungi concerned. Chapter XXI is headed "Diseases not Fully Investigated," and includes grey-spot of oats, die-back of elms and certain virus diseases. There follow a few pages devoted to general methods employed against plant diseases, an Appendix in which the fungous diseases are tabulated according to their host-plants, and a general index. The volume is illustrated by 399 figures in the text, three being the rather crudely coloured figures of rust fungi from the original edition.

In his preface the author states that he is "conscious of the numerous deficiencies of my book," and many of these deficiencies are so obvious that the author had no right to leave them uncorrected for the bewilderment and misleading of students. The numerous misprints are annoying; the errors of fact are serious, and the extent to which the mycoplasm hypothesis is pushed is almost ludicrous. The illustrations as a whole are poor, and some are probably as bad as any that have been published

in pathological literature; legends are occasionally inaccurate and, save for a rare "highly magnified," there is no indication of size. Each disease is followed by references and, looking at random through these, one finds that the latest citation for powdery mildew is 1904, for plum pocket 1895, chrysanthemum rust 1903, covered smut of barley 1888, downy mildew of cucumber 1904, sooty mould of cereals 1897, vine anthracnose 1905, and so on. Even on *Phytophthora* blight of potato the latest reference is 1919. But for a few comments and references which, one suspects, have been inserted by Mr Goodwin, the volume is almost entirely "pre-war" in its matter, points of view and appreciation of values, and in my opinion it would have needed complete revision by an up-to-date plant pathologist to have made the labour of translation in any way worth while. Finally the book costs 35*s*.

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